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Anticancer Effects of LBA-ST Yogurt Supernatant on HeLa Cells via Heat Shock Protein 27 Decrease *In Vitro*

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Abstract

Heat Shock Protein 27 (Hsp27) is overexpressed in cervical cancer as a response to stress conditions. Hsp27 overexpression increase invasion, migration, and adhesion pathways of cancer cells. The Yogurt supernatant contains Short-Chain Fatty Acids (SCFA) include acetate, lactate, and butyrate which have anticancer activity. This study aimed to investigate supernatant of LBA-ST (*Lactobacillusbulgaricus-acidophilus, Streptococcusthermophillus)* Yogurt can decrease the expression of Hsp27 in HeLa culture cells. The mechanism on how supernatant yogurt inhibit invasion, migration, and adhesion was studied by immunocytochemistry. The data was then collected and analyzed using One-Way ANOVA. From this study, it can be concluded that the expression of proteins that play a role in invasion, adhesion, and migration of the Hsp27 was proven to be decreased ($p< 0.05\pm0.005$).

Keywords: HeLa cells, yogurt supernatant, *Lactobacillus bulgaricus-acidophilus, Streptococcus thermophillus*, Hsp27.

INTRODUCTION

The growth of abnormal cells in mucosal servix, exocervix, and endocervix known as cervical cancer (American Cancer Society, 2012). Cervical cancer has the second highest prevalence of cancer in woman following breast cancer. Human papilloma virus (HPV) is the main cause in cervical cancer (Castellsagué, 2012).

Vaccination, surgery, radiotherapy, and chemotherapy are the common therapies in the cervical cancer. Those therapies has serious side effects, and promising solution has not been established yet. Moreover, vaccination still can not be implemented in every country because of the high cost and limited availability (Goldie, *et al*., 2005).

The fermentation products are recommended as the alternative therapy (Rahman, 2013), because of their multiple mechanisms of action, high safety, and the continuity of production (McFarldan, 2009). Yogurt is the product obtained from the milk fermentation by the lactic acid bateria. Short-Chain Fatty Acids (SCFAs) such as butyrate, acetate, and lactate are the dominant metabolite compound in yogurt *Lactobacillus bulgaricus-acidophillus* and *Streptococcus thermophillus* (LBA-ST) and were reported to have anticancer activity (Hara and Fergus, 2007).

Those metabolites could have antimutagenic, antioxidative, and anticarcinogenic effect, along with the modulation of immune system (Wollowski, *et al.*, 2001). In the identification of cervical cancer marker, Hsp27 is known as the critical role in the development of invasion, migration, and cell adhesion (Patel, *et al.*, 2002; Rerole, *et al.*, 2010) This study aim was to investigate LBA-ST yogurt supernatant in the decrease of Hsp27 expression on HeLa culture cells to inhibit the invasion, migration, and adhesion.

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MATERIALS AND METHODS

Fermentation of LBA – ST Yogurt (*Lactobacillusbulgaricus-acidophilus, Streptococcusthermophillus)*

One litre of milk was heated in the temperature of 82-100ºC. Then the temperature was cooled down to 42-44ºC. As many as 5 gr of yogurt stater (yoguormet t.m) containing LBA-ST were dissolved in a cup of the milk and then homogenized well by adding a litre of milk. Incubation were done in the temperature of 4ºC for 4.5 hours or untill the pH reach 4-5. Fermentation was then stopped by storing the yogurt in the refrigerator prior to usage (Yogourmet, 2014).

Preparation of supernatant, pellet, and whole LBA-ST yogurt

As many as four falcons of 50 mL LBA-ST Yogurt were centrifuged for 15 minutes in 4000 rpm. The supernatant were separated from the bacteria pellet by filtration with Whatman paper. The storage were done in the temperature of 4ºC.

HeLa Cell Culture

HeLa cells were obtained from *Laboratorium Pengujian dan Penelitian Terpadu* (LPPT), *Universitas Gadjah Mada, Yogyakarta*. HeLa cells were cultured in RPMI 1640 media supplemented with 10% (v/v) FBS and non essential amino acids. Cells were counted by haemocytometer. Cells were harvested with Trypsin-EDTA, and centrifuged in 1500 rpm for 8 minutes. The supernatant was removed and the pellet was resuspended in the added culture media. Cells were cultured in well plates and were incubated in the temperature of 37°C, 5% CO_2, and 100% humidity.

Hsp27 Immunocytochemistry

As many as 5 x 10^4 cells/1000 µL HeLa cells supension were cultured with RPMI 1640 culture media and distributed in 24-well plate on the cover slips and incubated for 24 hours. After the cells were confluent, cells were treated by the supernatant of LBA-ST yogurt with the concentration of 10, 20, and 40% (v/v). As for the control group, 1000 µL of culture medium was added.

After 24 hours incubation, the cover slips were washed by cold PBS 2 times, fixed by cold methanol for 15 minutes, and washed by the PBS once. Then the hydrogen peroxide as the blocking solution was added, followed by incubation for 10 minutes. The immunocytochemistry was done by adding 400 µL Hsp27 monoclonal antibody in 1:200 dilution. The incubation was done for 1 hour, then the cover slips were washed by the PBS. Biotinylated universal secondary antibody was then added. PBS were used to wash the cells before and after the incubation by streptavidin peroxidase enzyme. The incubation was done for 10 minutes. The chromogene substrate diaminobenzidine were added, continued with incubation for 10 minutes. Next, cells were washed by aquadest. As the counterstain, Meyer haematoxylin was added prior to incubation for 3 minutes, followed by washing using aquadest. The coverslips were then dried up by xylol and ethanol. The coverslips were placed on the microscope slides and mounted with entellan. Cells were observed and the Hsp27 expression were counted using the microscope.

Data Analysis

The data were analyzed statistically by the SPSS 16 for Windows 7 program. The normality and homogenity study were done first, and the analysis of the data were done by ANOVA (p = 0.05 and α = 0.05).

RESULTS AND DISCUSSION

The effects of LBA-ST yogurt supernatant to the expression of Hsp27

The aim of this study was to investigate the effects of LBA-ST yoghurt in the decrease of Hsp27 expression on HeLa cells. The entire result of this study showed that LBA-ST yoghurt has anticancer on HeLa cells. Anticancer activity showed by the data of the expression of Hsp27 in the Control cells (Fig. 1) and the Hsp27 expression index by the treatment of LBA-ST yoghurt supernatant (Fig. 2).

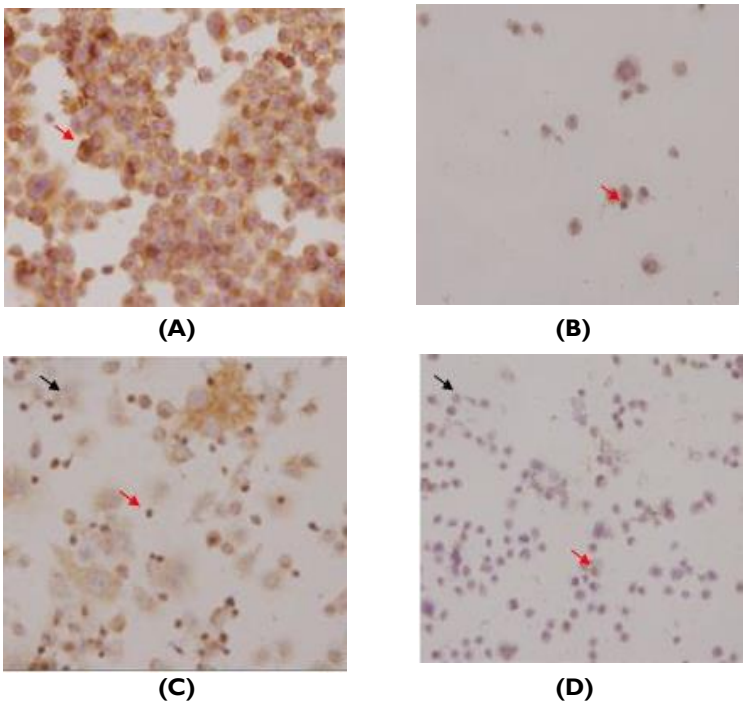

Figure 1. The expression of Hsp27 in the control cells (A), the treatment of supernatant of 10% (v/v) (B), 20% (v/v) (C), and 40% (v/v) (D) LBA-ST yogurt. Incubation was done for 24 hours, and the expression of Hsp27 in cells lead to the increase of the quantity of color and morphological changes in HeLa cells (400x magnification), decreased expression (→), overexpression (→). Distribution of cells and different cell sizes were obtained in the same magnification.

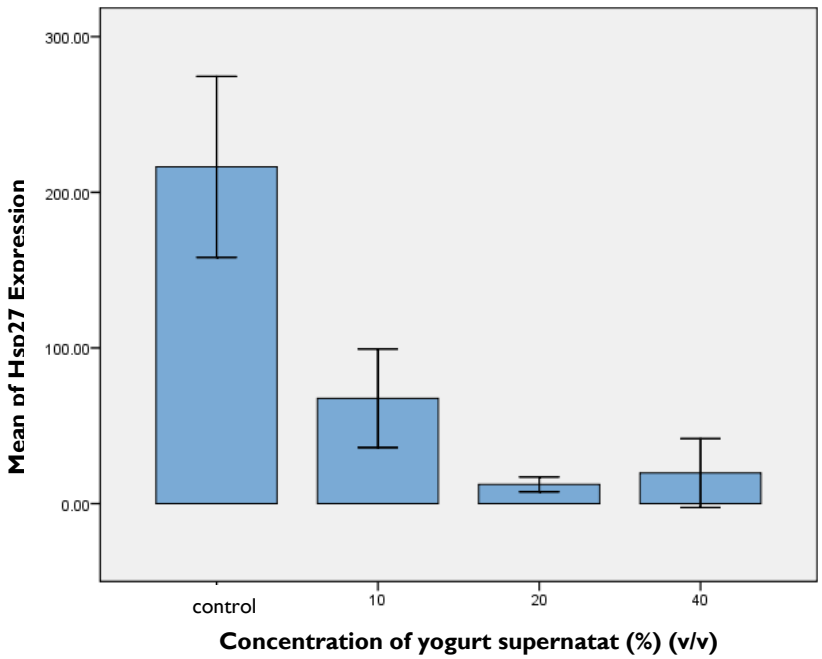

Figure 2. Hsp27 expression index by the treatment of LBA – ST yogurt supernatant in the concentration of 10%, 20%, dan 40% (v/v) compared to the control. The graph of reduction in Hsp27 expression were done by the data analysis by ANOVA (p<0.05 ± 0.005).

The testing of the anticancer mechanism in the cervical cancer can be done by the observation of Hsp27 expression. In this study, Hsp27 expression in HeLa cells were decreased by the treatment of supernatan of yogurt LBA significantly compared to the control (p<0.001). The highest reduction of Hsp27 expression was obtained by the treatment of supernatan of yogurt LBA in the concentration of 20% (v/v). Based on the result of this study, LBA-ST yogurt supernatant may inhibit the proliferation, migration, and invasion of cervical cancer cells, and also induce apoptosis.

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