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Research Article

Comparison on the Cancer Specific Cytotoxicity of Three Gingers (*Zingiber officinale* Rosc) Leaves Varieties from Indonesia

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ABSTRACT

This study was performed to get more insight the cancer specific cytotoxicity of ginger leaf (GL). Three GL varieties (Gajah, Emprit, and Red) were extracted and fractionated. Each etyl acetate fraction in concentration of 200 µg/ml was tested about specific cytotoxicity toward cancer dan normal cells. Cancer cell lines used in this study were human colorectal (HCT116) and human breast (MCF-7 and T47D) cancer while normal cell line was human fibroblast (KMST-6). Based MTS assay method, the results showed Gajah and Emprit GL more significantly reduce cell viability of HCT116 and T47D than Red GL although there was no difference on the efficacy of both varieties. All varieties of GL also significantly reduce cell viability of MCF-7 compare to PBS control. However, there were not significant differences between those GL varieties on their effectiveness against MCF-7. In contrast, there were no effects on the KMST-6 due to all GL varieties treatment compare with PBS control. All data suggested that GL treatment only inhibited in the cancer cells without detrimental effect in the normal cells. Effectiveness of GL against cancer cell varies depend on the varieties. Gajah and Emprit GL are better varieties possess the cancer specific cytotoxicity that merits to be developed as promising chemo preventive agent in the future.

Keywords: Cancer, Specific, Cytotoxic, Ginger Leaf, Varieties.

INTRODUCTION

Cancer is one of the leading causes of death worldwide. In 2012, death caused by cancer is approximately 8.2 million of the world population. According to the data of Health Ministry, cancer prevalence of Indonesia was 1: 1,000 in 2011 and in 2013 that number increased to 4: 1,000 people with the highest rate of incidence and mortality was cervical cancer, overtaken by breast and liver cancer[1]. Meanwhile, chemotherapy as one of standard option for cancer treatment still has the drawbacks in its application[2]. The effectiveness of Chemotherapy is inhibited by its toxicity toward normal tissue in the body[3,4]. Therefore, innovations to cure cancer disease with the low side effects always become an attractive goal along the journey of new anticancer discovery.

It has been known that food possesses the ability in prevention and medication of diseases since thousands years ago[5]. This concept is known as nutraceutical. One of herbal plants-Ginger (*Zingiber officinale Rosc*) has been being a part of various traditional healthy foods and beverage during this period. Ginger plant is quite abundant in availability, and Central Java of Indonesia is well known as herbal industry region able to produce rhizomes about 26 tons / year[6]. Gingers as perennial herbs belonging to the family Zingiberaceae have been widely used as spices, condiments and herbal medicine for treatment of cold, fever, headache, nausea and digestive problems[7]. Ginger and its general compounds such as gingerols, shogaols, paradols and zingerone exert immuno-modulatory, antiapoptotic, anti-tumourigenic, anti-inflammatory, antihyperglycaemic, anti-hyperlipidaemic, antioxidant and anti-emetic activities[7]. Besides their rhizomes, ginger leaves (GL) have also been used for food flavouring and traditional medicine[8].

Previous study reported that GL in the range dose of 100-200 µg/ml, effectively reduced human colorectal and breast cancer cells lines through transcription factor 3 (ATF3) activity- induced apoptosis mechanism[9]. Thus, in light of the anticancer mechanism of GL, this study was performed to get more insight the cancer specific cytotoxicity of GL. It emphasized that GL with different varieties in Indonesia such as Emprit, Gajah, and Red are only cytotoxic to cancer cell without any adverse effect to the normal cell.

MATERIALS AND METHODS

Preparation of GL extract

Three varieties of GL (Emprit, Gajah, and Red) were collected from the ginger farmer in the Central Java Province, Indonesia. 100 gram of GL powder was

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A B C

Figure 1: Rhizomes of three ginger varieties such are Gajah (A), Emprit (B), and Red (C) ginger.

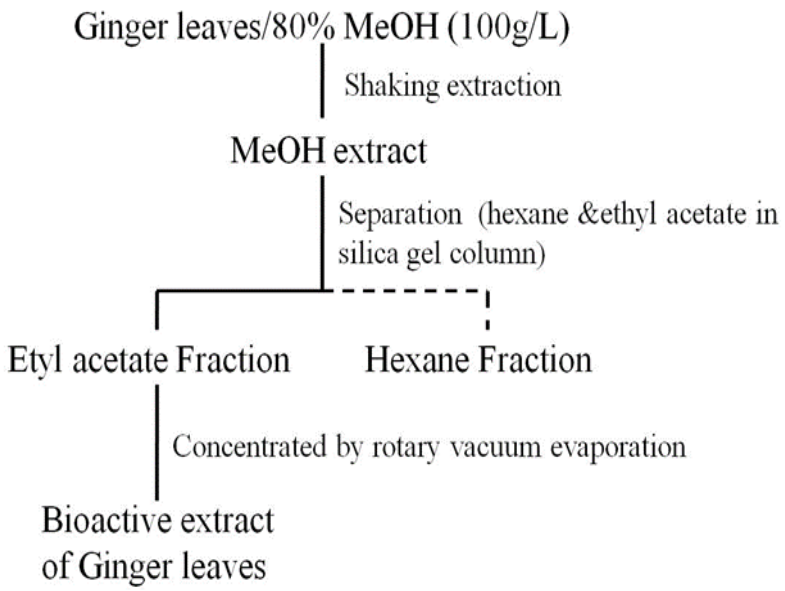

Figure 2: Flow chart diagram of separation schematic procedure of GL extract.

extracted with 1000 ml of 80% methanol with shaking for 24 h. After 24 h, the methanol soluble fraction was filtered and concentrated to approximately 20 ml volume using a vacuum evaporator and then fractionated with hexane and ethyl acetate in a separating funnel. The ethyl acetate fraction was separated from the mixture, evaporated by a vacuum evaporator, and prepared aseptically and kept in a refrigerator.

Cell culture and treatment

Human colorectal cancer cell line (HCT116), human breast cancer cell lines (MCF-7 and T47D), and human normal fibroblast (KMST-6) were purchased from the Japan cancer research source bank (JCRB, Ibaraki, Japan). Cells and sub culture were maintained according to the supplier's recommendation. Culture medium were DMEM, EMEM, and RPMI (medium according to the type of cell) supplemented with 10% fetal bovine serum (FBS). Cells were cultured under humidified atmosphere of 5% CO2 at 37°C. The extracts of ginger leaf (GL) were dissolved in dimethylsulfoxide (DMSO) and treated to cells. DMSO was used as a vehicle and the final DMSO concentration did not exceed 0.1% (v/v).

Cytotoxicity tests

The cytotoxicity of samples was determined by measuring the cell viability using MTS assay according to the manufacture's instruction (Cell Titer 96® AQueous non-radioactive cell proliferation assay, Promega Co, Madison USA). Briefly, the sample or vehicle was added to the 96-well plate, and dried aseptically for 30 min. Cells cytotoxicity titration curve was constructed with serial dilution of sample in a 96-well microplate. Cell suspended in the appropriate medium was seeded at $1x10^3$ cells (100µl) per well and incubated in humidified atmosphere, 5% CO_2 at 37˚C for overnight. The cell viability after the treatment was determined by MTS assay kit. All the experiments were performed in triplicate, and the cell cytotoxicity was expressed as the relative viability or living cell number of the sample-treated cells against untreated controls.

Statistical analysis

One-way ANOVA was used to identify the difference levels of SM for some indicated parameters. For group differences, post hoc multiple comparisons Duncan multiple range tests were used. Statistical analyses were performed using SPSS for Windows. $P < 0.05$ was considered statically significant[10].

RESULTS AND DISCUSSION

Chemotherapy as an option for cancer therapy still has weaknesses such severe side effects and dose-limiting toxicities[2]. Therefore innovation to cancer healing with the

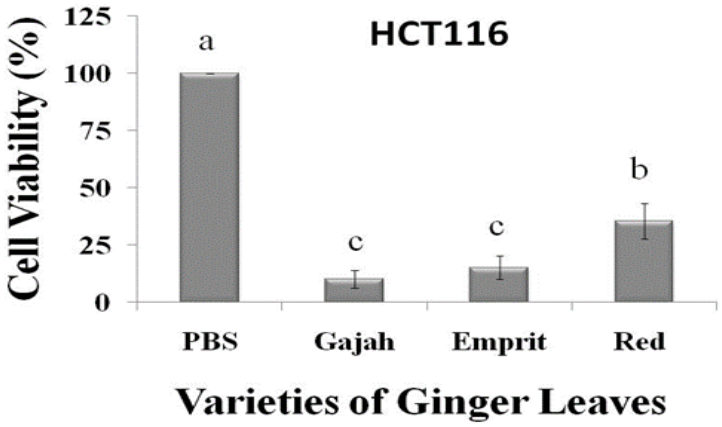

Figure 3: The effect of some GL varieties (200 µg/ml) on the cell viability of human colorectal cancer cells (HCT116). Data were expressed as mean of % cell viability ± STDEV. The different superscript on the bar graphic showed the significant differences ($p < 0.005$) between some treatment group (PBS control, Gajah, Emprit and Red varieties of GL).

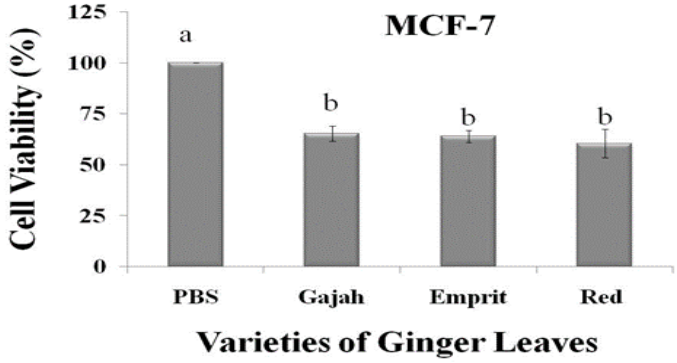

Figure 4: The effect of some GL varieties (200 µg/ml) on the cell viability of human breast cancer cells (MCF-7). Data were expressed as mean of % cell viability ± STDEV. The different superscript on the bar graphic showed the significant differences ($p < 0.005$) between some treatment group (PBS control, Gajah, Emprit and Red varieties of GL).

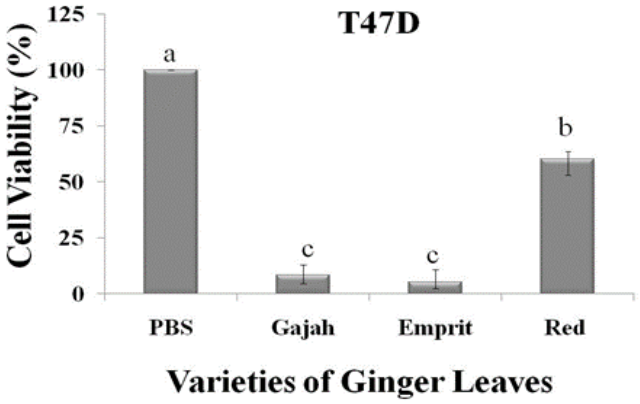

Figure 5: The effect of some GL varieties (200 µg/ml) on the cell viability of human breast cancer cells (T47D). Data were expressed as mean of % cell viability ± STDEV. The different superscript on the bar graphic showed the significant differences ($p < 0.005$) between some treatment group (PBS control, Gajah, Emprit and Red varieties of GL).

low side effects always become an important and interesting goal along the journey of anticancer discovery. The promising anticancer agent should possess certain cytotoxic feature. It means an agent is only toxic toward cancer cells without any adverse effect to normal cells. Through this study, we tried to evaluate the specificity of ginger leaves (GL) as anticancer agent. Previous studies has demonstrated the effectiveness of GL against various type of cancer cells such as human colorectal and breast cancer cell lines[8,9,11]. However, during this time data showed the effect of GL to the normal cells as manifestation of its safety has not been elucidated clearly. Recent study was conducted to evaluate the cancer specific cytotoxicity of GL by measuring cell viability of normal beside cancer cell line after treating with active fraction of GL. Moreover, this study also observed the comparison of cancer specific cytotoxicity of three GL varieties.

In this study we used three cultivar GL i.e. Emprit, Gajah, and Red. Those leaves are almost similar morphologically. The most striking differences among the three varieties can be observed on their rhizomes as showed in the Fig.1. Gajah ginger is the most widely cultivated in Indonesia. It has larger rhizomes, consumed in the form of processed and fresh with flavors that are not so spicy[12]. Emprit ginger is the dominant varieties planted as spice herbs, processed herbs and medicines in Indonesia. They have small segments of rhizomes with white color and flavor spicier[13]. Red Ginger is a variety that is quite rare compared to other varieties. It has reddish and smaller rhizome with the pungent smell, so often used for making ginger oil and pharmaceuticals[13]. In an attempt of their leaves utilization, three cultivar GL were extracted and fractionated as described in the Fig.2. Each etyl acetate fraction in concentration of 200 µg/ml was tested about specific cytotoxicity toward cancer and normal cells. Cancer cell lines used in this study were human colorectal (HCT116) and human breast (MCF-7 and T47D) cancer while normal cell line was human fibroblast (KMST-6).

Based MTS assay method, the results showed Gajah and Emprit GL extracts were more reduce effectively cell viability of HCT116 and T47D compare with Red GL although between Gajah and Emprit GL were not observed differences in their efficacy toward both cancer cells (Fig. 3 and Fig.5). In addition, all varieties of GL also significantly reduce cell viability of MCF-7 compare to PBS control (Fig.4). Unfortunately, there was not significant different between those GL varieties on their effectiveness against MCF-7 (Figure. 4). In the other hand, there were no effects on KMST-6 due to all GL varieties treatment compare with PBS control (Figure.6). All data suggested that GL treatment only inhibited in the cancer cells without detrimental effect in the normal cells. Thus, GL demonstrated the cancer specific cytotoxicity. Effectiveness of GL against the cancer cell showed variation among the varieties. This was probably influenced by the different level of bioactive composition contained in GL.

It has been known that medicinal components produced by plants usually are stored in their leaves[14]. Previous study reported that most important groups of secondary metabolite such as flavonoids and phenolics are contained in GL[8]. Level of both bioactive compounds in GL showed variation among their varieties[15]. Either flavonoids or phenolics, they have important roles in human life and health. Previous studies suggested that some flavonoids and phenolics could be able to control cancer cell growth in the human body[16-18]. However, all suggestions above are still needed further investigation in the future time. It is necessary to measure the kind of compound and their concentration levels vary in Gajah, Emprit, and Red GL.

In the other hand, susceptibility of cancer cells toward GL extracts likely was influenced by theirs histological characteristics. Two types of breast cancer cells in this study, MCF-7 and T47D showed the different response to GL treatment. In the same level of treatment (200 µg/ml) and varieties of GL (Gajah and Emprit), cell viability of T47D lower than MCF-7. The data suggest that T47D is more susceptible to GL treatment than MCF-7. It was known that characteristics of MCF-7 are resistance to the chemotherapy agent such doxorubicin, expression of estrogen receptor (ER), and over-expression of Bcl-2[19-23]. On the other hand, T47D possessed the expression of p53 protein mutation and doxorubicin sensitive[23-25]. Therefore, the different response observed in both cancer cells likelihood associate with the expression of estrogen receptor (ER) and the present of mutation on the tumor suppression gene (p53). There is notion that anticancer

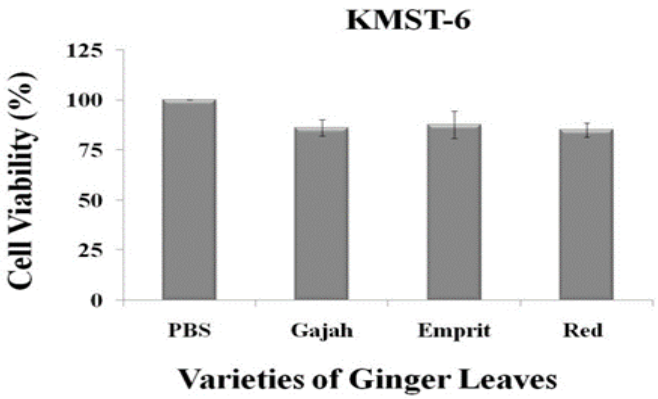

Figure 6: The effect of some GL varieties (200 µg/ml) on the cell viability of normal human fibroblast cells (KMST-6). Data were expressed as mean of % cell viability ± STDEV.

mechanism of GL, especially in combat to the breast cancer cells, not only involve the transcription factor 3 (ATF3) activity- induced apoptosis as described in the earlier study[9]. However, this statement still needs more contemplation that may be evidence in the future mechanism test of GL against cancer cells.

In addition, T47D was more sensitive than MCF-7 to GL treatment (Fig. 4 and 5) apparently have similar phenomenon with the doxorubicin sensitivity of both cancer cells. It was more surprising, although various cancer cells were quite sensitive, KMST-6 normal cells showed no detrimental effect to GL treatment (Fig.6). These results bring out the speculation that GL extracts especially Gajah and Emprit GL have better the cancer specific cytotoxicity than doxorubicin. There was report that doxorubicin application is clinically limited by cardiotoxicity effect in the long period of therapy[25]. Based on the facts GL extract is the promising anticancer treatment in the future. Taking into consideration that many cancer patients is sometime unsatisfied toward chemotherapy, GL extracts merits to be developed as anticancer functional food or nutraceutical that is not only healing cancer disease but also can improve the life quality of cancer patient accordingly.

CONCLUSION

Leaves extract of three ginger varieties i.e. Gajah, Emprit, and Red ginger exhibit the cancer specific cytotoxicity. Gajah and Emprit varieties found the better efficacy against colorectal (HCT116) and breast (T47D) cancer cells than another. Thus Gajah and Emprit GL is the promising anticancer agent in the future. Bioactive constituent responsible for the cancer specific cytotoxcity of GL should be contemplated in the further study as well as their mechanism of action.

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