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by Artikel 15 Cytotoxic Activity Of Ethanol Extract Of Arbuscula

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Original Article

Cytotoxic Activity of Ethanol Extract of Arbuscular Mycorrhizal Fungi Induced Ginger Rhizome on T47D Breast Cancer Cell Lines

Netty Suharty, Fatma Sri Wahyuni, Dachriyanus*

ABSTRACT

Objective: A study of investigate the cytotoxicity activity of ethanolic extract of ginger (*Zingiber officinale* Rosc.) induced with arbuscular mycorrhizal fungi (AMF) against T47D cells line breast cancer have been conducted. **Methods:** Cytotoxicity were determined using the "microtetrazolium (MTT) Assay", by measuring the activity of mitochondrial dehydrogenase in living cells that have ability to convert pale yellow of dissolved MTT to purple formazan product. The extract used at various concentration (0.1, 1.0, 10 and 100 µg / mL. The level of cytotoxic actifity was determined by calculating the inhibitory concentration (IC_{50}) value that was based on the precentage of cell death after 24 h treatment with the extract. The change of cell morphology were observed by using inverted microscope. **Results:** The statistic results proved that ethanol extract of AMF induced ginger rhizome could barriers T47D breast cancers significantly at concentrations of 10 µg / mL and 100 ug / mL, with IC_{50} value was 12.5 ± 3.73 µg / mL. centration of 0.1 µg / mL, 1.0 µg / mL, 10 µg / mL and 100 mg / mL. Results of statistical analysis showed that the ethanol extract of ginger rhizome induced AMF at a concentration of 10 µg / mL and 100 µg / mL was able to inhibit the growth of breast cancer cells T47D significantly. **Conclusion:** The results showed the ethanol extract of AMF induced ginger rhizome was potential as herbal medicine for cancer-related ailments with IC_{50} value was 12.5 ± 3.73 µg / mL.

Key words: Ginger, AMF, T47D, Breast cancer, Cytotoxicity, MTT assay.

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INTRODUCTION

Breast cancer is a major cancer commonly in women, were over one million women worldwide are diagnosed with this disease.[1] Although there are many therapeutic strategies including chemoteraphy to treat cancer, high systemic toxicity and drug resistence limit the successful outcames in most cases.[2] The other strategies are being developed to control and treat cancer, one such approach could be used the medicinal plants agents, would enhance efficacy while reducing toxicity to normal tissue.[3] Several Sumatran plants have been studied for their anticancer properties.[4-6] In this paper, cytotoxicity activity of of ginger (*Zingiber officinale* Rosc.) induced with arbuscular mycorrhizal fungi (AMF) against T47D cells line breast cancer was reported.

Ginger (*Zingiber officinale* Rosc.) family of zingiberaceae, has been widely used as a condiment though out the world for centuries. Ginger has been used as herbal medicine to tread a wide range of disorder such as anti inflamation, analgesic, dispepsia, nausea, vomiting, antibacteri, antioxidant and anticancer.[7-8] Ginger contains active phenolic components such as gingerol and shogaol which have antioxidant and anticancer effects.[9-10] Component carrier spicy ginger flavor that is gingerol, paradol, shogaol and zingerone have anti-inflammatory activity and chemopreventive effects of that shows prevention of cancer in experimental carcinogenesis.[11]

The present study uses ginger rhizome that has been induced by arbuscular mycorrhizal fungi (AMF), as one type of biological agent. The results of previous studies AMF may increase the resistance of plants to bacterial wilt disease, increase rhizome production and secondary metabolites from the rhizome of the ginger plant.[12] The aim of the study was to evaluate the cytotoxic effect of the ethanolic extract of AMF induced ginger on breast cancer T47D cell lines.

MATERIALS AND METHODS

Plant Material

The rhizome of ninth months AMF induced ginger were cultivated and collected from screen house of Herbal Medicinal Study Centre Andalas University Padang Indonesia.

Human breast cancer T47D cell lines was obtain from Tissue Culture Laboratory of Medicine Gajah Mada University, Yogyakarta, Indonesia).

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Chemicals

The chemical and solvents used in this experiment were ethanol, methanol (E grade) were obtained from Merck. Methil Thiazol Tetrazolium (MTT) was obtained from Sigma (Germany), Dimethyl sulfoxide (DMSO) and all solvents (AR grade).

METHODES

Extraction

The plant meterial was sliced and air dried in green house for 3 days, follow by oven drying at temperature 40°C for 24 h. The dry rhizomes were grinded to powder using laboratory grinder. About 2 kg of powdered the rhizome of ginger was macerated in 7 L of 70% ethanol for 3 days. This process repeated 3 times. The ethanol extract was evaporated and concentrated with rotary evaporator at 40°C.[13] the resulting extract was kept in the refrigerator. The extract is dried under reduced pressure by using rotary evaporator. Dried extract was dissolved in DMSO (Sigma) at stock solution of 100 mg/mL.

Cell Culture

Human breast cancer cell line T47D were cultured in RPMI with 10 % complete mudium (Gibco). The medium was supplemented with 10 % heat inactivated fetal bovine serum, penecillin G and streptomycin 100 µg/ml. The cell lines were maintained at 37° C in 5% CO_2 incubator.

Cytotoxic Assay and Cell Viability

Cells were seeded into 96-well plate (Nunc, Denmark) and precultured for 24 h, treated with ethanol extract of AMF induced ginger for 48 h. Cell Cytotoxicity was determined by MTT assay, were 20 µL extract at concentration 0.1 µg/mL; 1.0 µg/mL; 10 µg/mL; 100 µg/mL disolved in DMSO completely were added into 180 µL cell suspension in RPMI media. After 24 h incubation, added 20 µL MTT (Merc, Germany) reagent in phosphate buffer saline (PBS) to each well. The plate were incubated at 37°C, the medium was discharged and the purple percipitate wich had been formed in the cells were dissolved with 100 µL DMSO. The absorbance was measured at 550 nm wave length by Autoated Microplated Reader (Bio-Teck) and the cell death was calculated.[12] Cell viability was estimated by trypan blue dye exclusion. After 24 h incubation, the culture was observed under inverted microscope and morphological change of cells were identified.

Data Analysis

The relationship between the concentration of the test solution with cell viability shown in graphical form and the determined IC_{50} (concentration that inhibits 50% living cells) of the test solution. All experiments were repeated three times and the data were presented as the mean ± SD unless noted otherwise. Differences between data groups were evaluated for significance using one ways analysis of variance (ANOVA) followed by Duncan's multiple range test, using the software SPSS 14.0 for windows. P values less than 0.05 indicate statistical significance.

RESULTS

Citotoxicity test

Effect of Ethanol extract of AMF Induced ginger rhizome on T47D breast cancer cell line were determined by MTT Assay. The examined of the cytotoxicity effect of Ethanol extract of AMF Induced ginger in multiple concentration on human breast cancer T47D. The effective concentration was calculated from concentration-responce curve. The precentage of viability of each plate was shown in Table 1. Based on the MTT assay, it was found that Ethanol extract of AMF Induced ginger had

Table 1: Percentage viability of cells on each plate were treated with ethanol extract of AMF induced ginger.

Plate	Concentration			
	0.1 µg/mL	1.0 µg/mL	10 µg/mL	100 µg/mL
1	54.02 %	57.93 %	52.48 %	13.16 %
2	70.80 %	65.61 %	48.70 %	12.44 %
3	60.22 %	55.70 %	51.57 %	18.0 %
Average ± SD	61.68 % ± 12.1	59.74 % ± 7.9	50.91 % ± 4.9	14.53 % ± 4.2

Table 2: IC_{50} of Ethanol extract of AMF Induced ginger on breast cancer T47D Cells Line.

Factor	Rep. 1	Rep. 2	Rep. 3	Average of IC_{50}	SD
IC_{50}	15,25 µg/mL	8,25 µg/mL	14 µg/mL	12,5 µg/mL	±3,73

(The cell were treated with ethanol extract of AMF Induced ginger at various concentration for 24 h, the cell cytotoxicity was analyze by MTT assay)

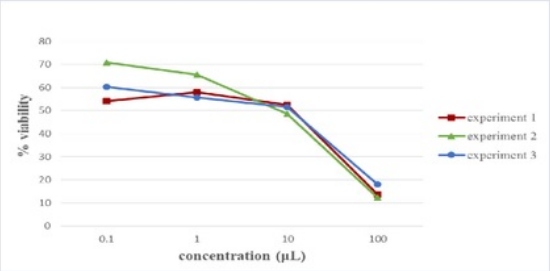

Figure 1: Dose- response relationship curve of the rhizome ginger ethanol extract with the percent viability of breast cancer cell T47D (24 h).

IC_{50} 12.5 ± 3.73 µL / mL (Table 2), dose-response relationship curve of the rhizomes of Ginger against breast cancer T47D cells (Figure 1).

Morphological changes evaluation upon treatment with extract

Figure 2 showed the difference picture when viewed with an inverted microscope, were difference concentration gives different morphological profile of cells when compared to control. At concentration 0.1 µg/mL and 1.0 µg/mL, many cells grow and attached at the base of the flask, cells grow very dense and very little distance between one cell to another cell, it almost no difference compared to control. In the other hand at concentration of 10 µg/mL, the cells look less than concentration of 0.1 and 1.0 µg/mL, cells are visible only in small groups and the distance a group of cells with other cells look far. In a concentartion of 100 µg/mL, the cells look less, many cells was dead, no cell nucleus be visible, it is also evident from the low absorbance value.

DISCUSSION

Ginger has been widely used as a condiment thoughout the world for centuries. It has been used as herbal medicine to tread a wide range of disorder such as anti-inflammation, antioxidant and anticancer.[3-4] Ginger root and its main phenolic compounds such as gingerols have anticarcinogenic activity, antioxidant and anti-inflammatory activity. Plant Materials used in the form of fresh samples of ginger plants induced

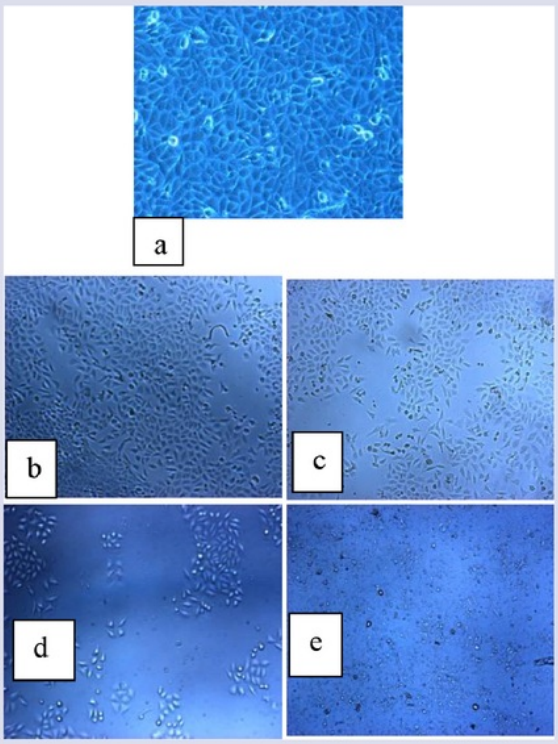

Figure 2: Morphological profile of the T47D cells after treated with ethanol extract of ginger 0.1 µg/mL (b), 1.0 µg/mL (c), 10 µg/mL (d) and 100 µg/mL (e) compared to control (a) for 24 h. (100 x enlargement).

by AMF, it has an activity increase of growth hormone ginger and chemical compound when compared with uninoculation plant.[12] T47D cancer cell cultures commonly used in cancer research *in vitro* because it has the ability in each cycle of cell replication and have a fast replication capability, high homogenity, that is suited for cytotoxic test.[12] T47D cancer cells are cells that have the function of the p53 gene is mutated, so it can not bind to p53 response elements on DNA. This results in reduced even loss of the ability of p53 gene to cell cycle regulation.

MTT assay is a colorimetric cytotoxic test method to determine the number of living cells based on changes in a solution of 3-(4,5-dimetil-thiazol-2-il)-2,5-difeniltetrazolium bromide were colored yellow to purple formazan crystals by active mitochondria in living cells.[12] Purple color intensity is directly propotional to the amount of the active cell metabolism. Formed the darker color, the higher the absorbance value and the more living cells.

The result of one Way ANOVA followed by Duncan Multiple Range Test (α=0.05), suggesting that the extract of ginger rhizome gave significantly different barriers to the growth of T47D breast cancer cells. The ethanol extract of ginger induced by AMF at a concentration of 100 µg / mL, had the highest activity inhibiting the growth of cancer cells T47D significantly different from the concentration of 10 µg / mL, 1.0 µg/mL. But the concentration of 1 µg / mL and 0.1 µg / mL was not significantly different (Table 1). The difference can be seen from the color produced by each concentration on the test plate. The color produced at a concentration of 100 µg / mL is slightly closer to purple or clear. When compared with a concentration of 0.1 µg / mL of concentrated purple and almost the same as the control. Purple formazan crystals produced as a result. Formazon crystals can penetrate the cell membrane and accumulates in the cells that are still alive. The data obtained that at a concentration of 100 µg / mL, significanly different from the concentration 10 µg / mL which when viewed on an inverted microscope many cells die, and many are the remains or carcasses from these cells, compared with a concentration of 1 µg / mL and 0.1 µg / mL were not different.

CONCLUSION

The inhibitory concentrations (IC_{50}) of ethanol ginger extract on T47D breast cancer cells was 12.5 ± 3.73 µg / mL. These results suggest that ginger has a cytotoxic activity that is characterized by a decrease in the percentage of T47D breast cancer cell viability in a highly significant (p <0.05).

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CONFLICT OF INTEREST

Author do not have conflict of interest.

ABBREVIATIONS

AMF: Arbuscular Mycorrhyzal Fungi; **MTT:** 3-(4, 5-dimethylthiazol-2, 5-diphenyltetrazoliumbromide; **IC:** Inhibition of Concentration.

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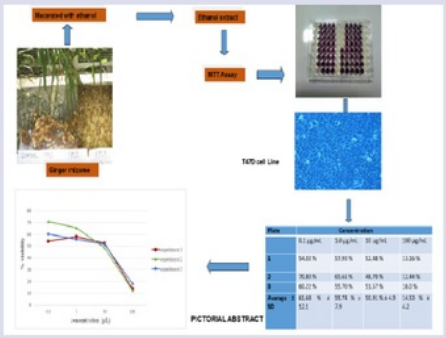
GRAPHICAL ABSTRACT



SUMMARY

The IC_{50} value of ethanol extract of AMF induced ginger rhizome is 12.5 ± 3.73 ug/mL could barriers T47D breast cancers cell line. The results showed the ethanol extract of AMF induced ginger rhizome was potential as herbal medicine for cancer-related ailments.

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Netty Suhatri got her undergraduate degree from Andalas University in 1984 and finished my PhD from Andalas University in 2010. Her research is in activity studies of Sumatran Plants especially Ginger

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