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 "microtetrizolium (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 activity was determined by calculating the inhibitory concentration (IC₅₀) 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 μg / mL, with IC₅₀ 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 IC₅₀ value was 12.5 ± 3.73 μg / mL. Key words: Ginger, AMF, T47D, Breast cancer, Cytotoxicity, MTT assay.

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 chemotheray to treat cancer, high systemic toxicity and drug resistance limit the successful outcomes 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 inflammation, analgesic, dispepsia, nausea, vomiting, antibacteri, antioxidant and anticancer.7-8 Ginger contains active phenolic components such as gingerol and shogoal 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.

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 material was sliced and air dried in a greenhouse for 3 days, follow by oven drying at temperature 40°C for 24 h. The dry rhizomes were ground into 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. 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, penicillin G and streptomycin 100 μg/ml. The cell lines were maintained at 37°C in 5% CO₂ 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 dissolved 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 precipitate which 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. 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 IC₅₀ (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 way 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 percentage 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 IC₅₀ 12.5 ± 3.73 μL / mL (Table 2), dose-response relationship curve of the rhizomes of Ginger against breast cancer T47D cells (Figure 1).

**DISCUSSION**

Ginger has been widely used as a condiment through the world for centuries. It has been used as herbal medicine to treat a wide range of disorder such as anti-inflammation, antioxidant and anticancer. 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...
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; 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, significantly 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 (IC₅₀) 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 Mycorrhizal Fungi; MTT: 3-(4, 5-dimethylthiazol-2, 5-diphenyltetrazoliumbromide; IC: Inhibition of Concentration.

REFERENCES

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.

ABOUT AUTHORS

**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.

**Fatma Sri Wahyuni** got her undergraduate degree from Andalas University in 1998 and finished my PhD from University Putra Malaysia in 2010. Her research is in activity studies of Sumatran Plants especially Genus Garcinia.

**Dachriyanus** got his undergraduate degrees from Andalas University in 1991 and finished my PhD from University of Western Australia in 1999. He is positioned as Professor of Pharmacy at Faculty of Pharmacy, Andalas University. His research is in chemical and biological activity studies of Sumatran Plants especially Genus *Garcinia* and *Rhodomyrtus tomentosa*.