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Brazilein Increased Cytotoxic Activity of Doxorubicin on MCF-7/DOX Cells

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Abstract

Brazilein is a compound obtained in a large amount from the dried heartwood of Secang (*Caesalpinia sappan* L.). Brazilein has strong cytotoxic effect in several cancer cell lines. This research was designed to evaluate the cytotoxic effect of brazilein and its combination with a chemotherapy agent, doxorubicin on MCF-7/DOX breast cancer cells. In the cytotoxicity assay, MCF-7/DOX cells were cultured in the presence of brazilein solely and in combination with doxorubicin for 24 hours and cell viability was evaluated by using MTT assay. MTT assay showed a dose-dependent inhibition of cell proliferation with IC_{50} value of 37 μM. Brazilein increased doxorubicin's cytotoxic activity on MCF-7/DOX cells. Both of single treatment with different concentration of brazilein 12.5 and 25 μM or doxorubicin 0.8 and 1 μM gave cell viability percentage above 80%, but combination of them led to decrease the cell viability percentage significantly. Based on this research, it can be concluded that brazilein is potential to be developed as a co-chemotherapy agent on breast cancer cell that have been resistant to doxorubicin. Futher study must be held to evaluate its molecular mechanism.

Keywords : brazilein, doxorubicin, MCF-7/DOX, cytotoxic.

INTRODUCTION

Breast cancer is the first leading cause of cancer-related-deaths among women worldwide (ACS, 2012). The high mortality rate indicates that chemotherapy has not been able to overcome cancer disease. One of the chemotherapeutic agents that is common to be used in breast cancer therapy is doxorubicin. However, several problems come up after the use of doxorubicin as a chemotherapeutic agent, such as its toxicity to normal tissues, severe side effects, and developed resistance. The side effects that usually arise are cardiomyopathy, congestive heart failure, and immunosupression. Hence, strategies and development of breast cancer treatment should be pursued. One strategy that will be evaluated in this research is the development of phyto-chemicals to inhibit cancer cells' growth as a chemopreventive agent and to reduce the problem faced in cancer treatment with chemotherapy, especially in breast cancer.

There are a lot of medicinal plants that are potent to be used as chemopreventive agent. One of them is secang (*Caesalpinia sappan* L.). Secang has been traditionally used as coloring agent in foodsand beverages. Besides, secang has a lot of pharmacological effects, especially in cancer. Several phenolic compounds are isolated from *C. sappan*, such as homoisoflavonoid protosappanin A, protosappanin B, 4-O-methylsappanol, caesalpin J, brazilin, and brazilein (Lim, *et al*., 1997). Brazilin and brazilein are the major compound of this plant proven to be responsible for its cytotoxic effect on cancer. Brazilein and brazilin (Fig. 1) have cytotoxic effect on lung cancer cells, nasopharyngeal cancer cells, and prostate cancer cells with IC_{50} value of 5-18 μM (Yen, *et al*., 2010).

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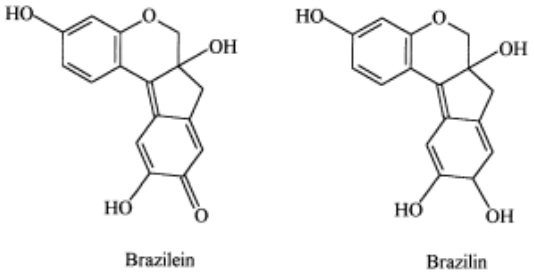


(A) **(B)**

Figure 1. Brazilein (A) and brazilin (B)

Brazilein inhibits survivin protein and induces apoptosis in HepG2 hepatocellular carcinoma cells (Zhong, *et al*., 2009). This study was conducted to evaluate the cytotoxic effect of brazilein, both alone and in combination with doxorubicin on MCF-7/DOX cells by using MTT assay.

MATERIALS AND METHODS

Sample Collection

Brazilein was isolated from secang (*Caesalpinia sappan* L.). Secang was obtained in the form of dried heartwood powder from Balai Besar Penelitian dan Pengembangan Tanaman Obat dan Obat Tradisional (BBPPTOOT) in Tawangmangu, Indonesia.

Cell Culture

The MCF-7/DOX cells was obtained from Cancer Chemoprevention Research Center (CCRC). The cells were routinely cultured in DMEM supplemented with 10% Fetal Bovine Serum (FBS) (Sigma-Aldrich, USA) at 37°C in a 5% CO_2 atmosphere, 1% penicillin-streptomycin, and 0.5 % fungizone. Subcultures were obtained after treatment with 0.05% trypsin (Gibco, Auckland) in phosphate buffer saline (PBS).

Cytotoxic assay

Exponentially growing cells were seeded on 96-well plates at 1×10^4 cells per well and incubated for 24 hours prior to addition of drugs. Test compounds were initially dissolved in DMSO or H_2O to make stock solution and then diluted with medium. Following a 24 hours incubation at 37°C, 5% CO_2, 100 µL of various concentrations of brazilein were added in each well in triplicates and cells were further incubated for 24 hours.

After 24 hours of incubation at 37°C, the medium was removed, and 100 µL of MTT reagent (1 mg/mL) in medium was added to each well. The plates were incubated at 37°C for 4 hours. At the end of the incubation period, the supernatant was removed, 10% SDS 0.01N HCL (100 µL) was added to each well, and plates were shaken gently for 15 minutes. After an overnight incubation at 37˚C, the metabolized MTT product dissolved was quantified by reading the absorbance at λ 595 nm using an ELISA reader (Bio-Rad). Absorbance was then calculated in order to get the number of viable cells. To determine cell viability, percentage of cell viability was calculated as:

$$\%cell\ viability = \frac{\text{abs of drug treated sample}}{abs\ of\ control} \times 100\%$$

The IC_{50} value is defined as the drug concentration required to inhibit cells growth by 50% of the control value.

RESULT AND DISCUSSION

Effect of Brazilein on MCF-7/DOX Cells Growth

The cytotoxicity of brazilein on MCF-7/DOX cells was determined by using MTT

assay. The IC_{50} value was 37 µM (Fig. 2). The MTT assay result demonstrated that brazilein had cytototoxic effect in MCF-7 cells. This effect was supported by the morphological change such as shrunken cell nuclei and membrane blebbing in some cells. The cytotoxic effect of brazilein in combination with doxorubicin must been held to evaluate the potency of brazilein as co-chemoterapy agent.

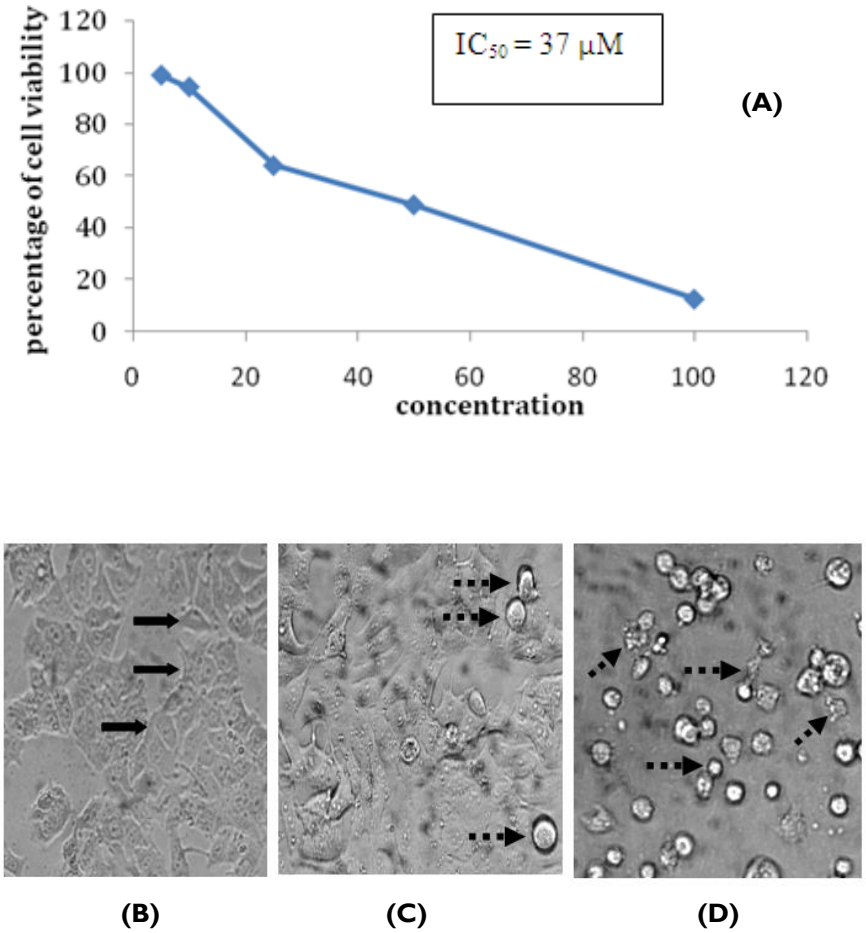


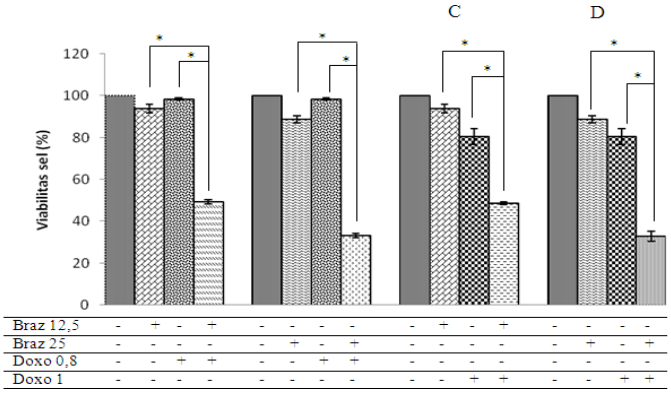
Figure 2. The cytotoxic effect of Brazilein on MCF-7/DOX cells. MCF-7/DOX cells (1×10^4 cells/well) were seeded on 96 wells plate. The cells were treated with brazilein for 24 h. After 24 hours, cells were added by MTT reagent to calculate the absorbance which represent viable cells. (A) Diagram of MCF-7/DOX cells viability after 24 hours brazilein treatment. MCF-7/DOX cells morphology of (B) cell control; (C) after 24 hours 25 µM brazilein treatment; (D) 50 µM brazilein treatment. Observation was done by using converted microscope with 400 x magnification. Cell viability profile was shown from average ± standard of error (SE) of 3 experiment. The normal cell morphology showed by the bold arrow (➡), meanwhile the change in cell morphology showed by the thin arrow (▪▪▶).

Brazilein increased doxorubicin's cytotoxicity on MCF-7/DOX cells

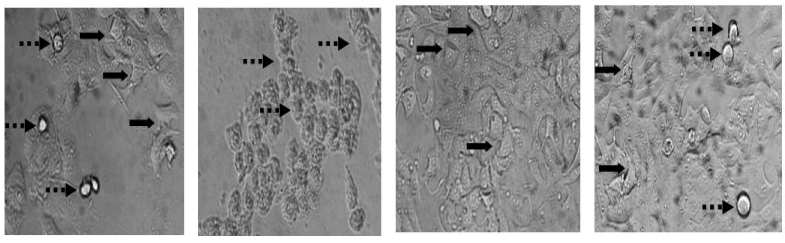
In order to assess the increasing of doxorubicin's citotoxicity by using brazilein as co-chemotherapy agent in MCF-7/DOX cells, the combinational cytotoxicity assay was conducted by using MTT assay. ANOVA test (p>0.05) showed that the cytotoxic effect between brazilein and doxorubicin alone and in combination had significant difference. MTT assay result showed that brazilein increased the cytotoxic activity of doxorubicin on MCF-7/DOX cells (Fig. 3).

Brazilein combination with doxorubicin on MCF-7/DOX cells treatment showed increased the sensitivity of the cells. Strong efficacy of brazilein in enhancing doxorubicin's cytotoxicity may occur through several molecular mechanisms, one of them is by the apoptosis induction mechanism.

It has already been reported that the cytotoxic effect of brazilein on Hep G2 cells occured via apoptosis induction and survivin protein expression suppression (Zhong, *et al.*, 2009).



(A)



(B) **(C)** **(D)** **(E)**

Figure 3. The cytotoxic effect of combination between Brazilein and Doxorubicin on MCF-7/DOX cells. MCF-7/DOX cells (1×10^4 cells/well) were seeded on 96 wells plate. The cells were treated with brazilein for 24 h. After 24 hours, cells were added by MTT reagent to calculate the absorbance which represent viable cells. Diagram of combination cytotoxic effect of brazilein and doxorubicin (A). MCF-7/DOX cells morphology after 24 hours brazilein treatment, control cell (B); Brazilein 25 µM (C); Doxo 1 µM (D); Brazilein 25 µM + Doxo 1 µM (E) (➡) normal cell morphology; (▪▪▶) morphology cell changing.

Long term use of doxorubicin causes severe side effect such as toxicity in normal cells and cancer resistance. It is most desirable to have more effective treatment by finding co-chemotherapeutic drugs. Co-chemotherapy is a cancer therapy strategy by combining natural agent or synthetic product with chemotherapeutic drugs. This strategy may reduce the side effect and toxicity of drugs. Brazilein is one of the natural agents that had been isolated from ethyl acetate fraction of Secang (*Caesalpinia sappan* L.) by vacuum colom chromatography, yielding reddish crystal.

Brazilein inhibits MCF-7/DOX cells' proliferation with IC_{50} value of 37µM. According to Teng, *et al*. (2005), compounds with IC_{50} values below 50 µM had a potent cytotoxicity against cancer cells, meaning that brazilein performed as a potent chemopreventive agent. The cytotoxic effect of brazilein on MCF-7/DOX cells might be caused by cell cycle arrest or apoptosis induction. Therefore, futher study must be conducted to evaluate the molecular mechanism that contribute to brazilein's potency as chemopreventive agent against MCF-7/DOX cells by using *in vitro* assay. Based on CCRC unpublished data (2012), on HeLa cervical cancer and WiDr colorectal cancer cells, brazilein's IC_{50} value were 61 µM and 243 µM, respectively.

The different potency of brazilein in different cells is related to the characterization of each cell. MCF-7 cells were characterized with overexpression of Bcl-2 and mutated caspase 3. In HeLa cells, p53 was inactive because of degradation, while overexpression of COX-2 is the characterization of WiDr cells. Futher study had been done to investigate the increasing cytotoxic activity of doxorubicin using brazilein as co-chemoterapeutic agent by MTT assay.

Brazilein demonstrated strong efficacy as co-chemotherapeutic agent when combined with doxorubicin. Lower doses of doxorubicin being used in combination with brazilein gave cytotoxic activity as potent as the doses used in single cytotoxicity assay. Different mechanism of doxorubicin and brazilein contributes to the combinational cytotoxic effect of both of them.

Doxorubicin interacts with DNA by intercalation and inhibits DNA topoisomerase II. Based on previous study, brazilein was cytotoxic on MCF-7 cells by inducing apoptosis through suppression of survivin protein expression (Tao, *et al*., 2011). Survivin is the smallest member of the mammalian IAP (Inhibition of Apoptosis) family that regulates cell death and cell cycle arrest (Fortugno, *et al*., 2002; Altieri, 2006). These molecular mechanism might be occurred by suppression of survivin protein expression through inhibition of its upstream protein, HER-2 and IKK. Inhibition of HER-2 and IKK could suppress the Pgp expression that correlated to resistance breast cancer cell induced by chemotherapeutic agent, doxorubicin (Siddiqa, *et al*., 2008; Gilmore, 2006).

The overall results showed the combination of doxorubicin and brazilein have a potency for breast cancer therapy especially in MCF-7 cells that have been resistant to doxorubicin. Effectiveness of combination therapy between brazilein and doxorubicin increased sensitivity of doxorubicin and induction apoptosis mechanism. Brazilein can be development as an co-chemotherapy agent with doxorubicin. Futher study must be established to evaluate the molecular mechanism of brazilein.

CONCLUSION

Brazilein was potential as doxorubicin co-chemotherapeutic agent on MCF-7/DOX breast cancer cells with the IC_{50} value of 37 µM.

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REFERENCES

Altieri, D.C., 2003, Validating Survivin as a Cancer Therapeutic Target, *Nat. Rev. Cancer*, **3**(1), 46-54.

American Cancer Society (ACS), 2011, *Breast Cancer Facts & Figures 2011-2012*, Atlanta: American Cancer Society, Inc.

Fortugno, P., Wall, N.R., Giodini, A., O'Connor, D.S., Plescia, J., Padgett, K.M., *et al*., 2002, Survivin Exists in Immunochemically Distinct Subcellular Pools and is Involved in Spindle Microtubule Function, *J. Cell Sci*., **115**(3), 575-585.

Gilmore, T. D., 2006, Introduction to NFkB: Players, Pathways, Perspectives, *Oncogene*, **25**(51), 6680-6684.

Lim, D.K., Choi, U., and Shin, D.H., 1997, Antioxidative Activity of Some Solvent Extract from Caesalpinia sappan Linn, *Korean J. Food Sci. Technol*., **28**(1), 77−82.

Siddiqa, A., Long, L. M., Li, L., Marciniak, R.A. and Kazhdan, I., 2008, Expression of HER-2 in MCF-7 Breast Cancer Cells Modulates Anti-Apoptotic Proteins Survivin and Bcl-2 Via The Extracellular Signal-Related Kinase (ERK) and Phosphoinositide-3 Kinase (PI3K) Signaling Pathways, *BMC Cancer*., **8**, 129.

Tao, L.Y., Li, J.Y. and Zhang, J.Y., 2011, Brazilein Induced Cells Apoptosis in Human Breast Cancer MCF-7 and Its Mechanism, *J. Sun Yat-Sen Univ*., **32**, 449-453.

Teng, W.Y., Huang, Y.L., Shen, C.C., Huang, R.L., Chung, R.S, and Chen, C.C., 2005, Cytotoxic Acridone Alkaloids from Te Stem Bark of Citrus Maxima, *J. Chinese Chem. Soc*., **52**(6), 1253-1255.

Yen, C., Goto, K.N., Hwang, T.S., Wu, P.C., Natschke, S.L.M., Lai, W.C., *et al.,* 2010, Total Synthesis and Evaluation of Brazilein and Analogs as Anti-inflammatory and Cytotoxic Agents, *Bioorg. Med. Chem. Lett*., **20**(3), 1037–1039.

Zhong, B., Wu, Y.J., Pan, S. and Zheng, 2009, Brazilein Inhibits Survivin Protein and mRNA Expression and Induces Apoptosis in Hepatocellular Carcinoma HepG2 Cells, *Neoplasma*, **56**(5), 387-392.