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RESEARCH ARTICLE

Antagonic Effect of Soursop Leaf Aqueous Extract and Doxorubicin Combination in MCF7 and T47D Breast Cancer Cell

Miranti Kania Dewi,[1] Siti Annisa Devi Trusda,[2] Lelly Yuniarti[3]

[1]Department of Pharmacology, Faculty of Medicine, Universitas Islam Bandung, Bandung, Indonesia,
[2]Department of Histology, Faculty of Medicine, Universitas Islam Bandung, Bandung, Indonesia,
[3]Department of Biochemistry, Nutrition, and Biomolecular, Faculty of Medicine, Universitas Islam Bandung, Bandung, Indonesia

Abstract

The success of breast cancer therapy is still not optimal and the side effects caused by breast cancer therapy. The use of standard drug combinations with herbs is often used as co-chemotherapy and is believed to increase the drug's effectiveness. However, research on the antagonistic effect of the drug combination is still minimal. This study examines the anticancer effect of soursop leaf aquoxes extract and the combined impact of doxorubicin on MCF7 and T47D breast cancer cells. This research is pure in vitro experimental study of MCF7 and T47D breast cancer culture cells at the Parasitology Laboratory of the Faculty of Medicine, Universitas Gadjah Mada in August 2018. Toxicity tests were carried out using the method of tetrazolium 3-(4,5-dimethylthiazol-2-yl) 2,5-diphenyltetrazolium bromide (MTT) to calculate cell viability. The IC_{50} value was obtained by analyzing probit regression calculation using SPSS software. The synergism of this compound with doxorubicin was determined based on the value of the Combination Index (CI) using a combination test with series 1/2 IC_{50}, 3/8 IC_{50}, 1/4 IC_{50}, and 1/8 IC_{50} and the data was analyzed using Compusyn 1.0 software. In this study, the effect of soursop leaf preparations will be tested on T47D and MCF7 breast cancer cell cultures and assess the impacts of co-chemotherapy of soursop leaf aqueous extract with doxorubicin. This study showed that IC_{50} soursop leaf aqueous extract in T47D breast cancer culture was 84 μg/mL and in MCF7 166.5 μg/mL. In contrast, the combined test showed that soursop leaf aqueous extract was antagonistic with doxorubicin in both T47D and MCF7 cancer cell cultures.

Keywords: Antagonic effect, breast cancer, doxorubicin, MCF7, soursop leaf, T47D

Efek Antagonis Kombinasi Extrak Air Daun Sirsak dan Doksorubisin pada Kultur Sel Kanker MCF7 and T47D

Abstrak

Keberhasilan terapi kanker payudara saat ini masih belum optimal dan terdapat efek samping yang ditimbulkan dari terapi kanker payudara tersebut. Penggunaan kombinasi obat standar dengan herbal sering digunakan sebagai ko-kemoterapi dan diyakini dapat meningkatkan efektivitas obat, tetapi penelitian mengenai efek antagonis kombinasi obat masih sangat terbatas. Penelitian ini mengkaji efek antikanker ekstrak air daun sirsak dan kombinasinya dengan doksorubisin pada sel kanker payudara MCF7 dan T47D. Penelitian ini merupakan eksperimental murni secara *in vitro* pada sel kanker payudara MCF7 dan T47D di Laboratorium Parasitologi Fakultas Kedokteran Universitas Gadjah Mada periode Agustus 2018. Uji toksisitas dilakukan menggunakan metode *tetrazolium 3-(4,5-dimethylthiazol-2-yl) 2,5-diphenyltetrazolium bromide* (MTT) untuk menghitung viabilitas sel. Nilai IC_{50} didapatkan melalui analisis menggunakan perhitungan regresi probit menggunakan perangkat lunak SPSS. Efek sinergis senyawa ini dengan doksorubisin ditentukan berdasar atas nilai Indeks Kombinasi (IK) menggunakan uji kombinasi dengan seri 1/2 IC_{50}, 3/8 IC_{50}, 1/4 IC_{50}, dan 1/8 IC_{50} serta data dianalisis menggunakan perangkat lunak Compusyn 1.0. Efek sediaan daun sirsak pada penelitian ini akan diujikan terhadap kultur sel kanker payudara T47D dan MCF7 serta menilai efek ko-kemoterapi ekstrak air daun sirsak dengan doksorubisin. Hasil penelitian ini menunjukkan bahwa IC_{50} ekstrak air daun sirsak pada kultur sel kanker T47D adalah 84 μg/mL dan pada kultur sel kanker MCF7 166.5 μg/mL, sedangkan uji kombinasi memperlihatkan bahwa ekstrak air daun sirsak berefek antagonis dengan doksorubisin pada kultur sel kanker T47D dan MCF7.

Kata kunci: Daun sirsak, doksorubisin, efek antagonis, kanker payudara, MCF7, T47D

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Correspondence: Dr. Lelly Yuniarti, S.Si., M.Kes. Department Biochemistry, Nutrition and Biomolecular, Faculty of Medicine, Universitas Islam Bandung. Jln. Tamansari No. 22, Bandung 40116, West Java, Indonesia. E-mail: lelly.yuniarti@gmail.com

Introduction

Breast cancer is the most common cancer in women worldwide and second only to lung cancer as a most cancer-related killer in developed countries.[1] Breast cancer is a significant public health problem with estimated new cases worldwide, reaching 1,384,155 and nearly 459,000 deaths. It predicted that the incidence of breast cancer worldwide would earn around 3.2 million new points per year by 2050.[2] Breast cancer is the disease that most often raises new cancer cases in most countries (154 countries). Indonesia is included in 154 countries when viewed from the 2018 global statistical cancer pattern; there are approximately 2.9 million new breast cancer cases with a total mortality rate of 620 thousand.[3]

The success rate of breast cancer therapy has not been 100% certain to eliminate cancer, plus the side effects that arise can cause patients not to conform to treatment. Research is needed for alternative medicine that is more effective than previous treatments and has more minimal side effects.[3,4] Besides, several studies have shown that Indonesian people have more confidence in herbal medicines because herbal medicines are inherited based on the experience of parents in using these drugs, giving rise to a sense of trust in them.[5,6]

Indonesia is rich in natural ingredients that can prevent and treat cancer, one of which is soursop (*Annona muricata* L.), a family of Annonaceae. Soursop contains acetogenin, tannins, flavonoids. Acetogenin has a selective cytotoxic effect on cancer cells and multidrug-resistant cancer cells with minimal toxicity to healthy cells. Flavonoids have a metastatic inhibiting impact on the culture of breast, liver, colon, lung, and ovarian cancer cells. Tannins can inhibit the growth and angiogenesis of Caco-2 colon cancer cells; tannin derivatives have a selective cytotoxic effect on cancer cells by inducing apoptosis.[7–9]

One cancer treatment strategy to reduce side effects is to use a drug combination. One of the advantages of using various drugs is to increase the efficacy of the therapeutic effect. It also reduces the dose but increases or maintains the same effectiveness to avoid toxic effects. It also minimizes or slows drug resistance occurrence and is selective synergy/synergy efficacy (deadly synergy on cancer cells but not in normal cell host).[10–12] However, the combination of drugs or the use of drugs simultaneously does not always provide a synergistic effect. Interaction of drug combination can also produce an antagonistic response due to the opposite effect of each drug.[13] A drug interaction is defined as the "pharmacologic response" of a drug administered concomitantly with another substance that can alter the patient's response to the drug. Consequences of changes in response or reactions from drug-drug interactions (DDI) can be associated with decreasing in drug effect.[14] The purpose of this study was to look at the anticancer and co-chemotherapy activities of soursop leaf aqueous extract against breast cancer cells.

Methods

This study is an in vitro experimental study of MCF7 and T47D breast cancer cultures. The research activity was carried out at the Parasitology Laboratory of the Faculty of Medicine, Universitas Gadjah Mada Yogyakarta, in August 2018. The anticancer test was carried out using the tetrazolium 3-(4,5-dimethylthiazol-2-yl) 2,5-diphenyltetrazolium bromide (MTT), and cell viability was measured using an ELISA reader with wavelength λ=550–600 nm (595 nm). The test was carried out at soursop leaf aqueous extracts (SLAE) concentration series 250 μg/mL, 125 μg/mL, 62.5 μg/mL, 31.25 μg/mL, 15.625 μg/mL, 7.8125 μg/mL, and 3.906 μg/mL. Whereas doxorubicin was carried out in series of 100 μg/mL, 50 μg/mL, 25 μg/mL 12.5 μg/mL, 6.25 μg/mL, 3.125 μg/mL, and 1.56 μg/mL. Concentrations that can inhibit 50% of cells (IC_{50}) are determined using probit regression calculations using SPSS software. Then the determination of synergism is measured using a combination test with the concentrations series ½ IC_{50}, 3/8 IC_{50}, ¼ IC_{50}, and 1/8 IC_{50}, both SLAE and doxorubicin. Cell viability was determined using an ELISA reader with a wavelength of λ=550–600 nm (595 nm). Combination Index (CI) values were analyzed using Compusyn 1.0 software with the toxicity criteria based on Baharum et al.,[15] namely potent (20 μg/mL), moderate (>20–100 μg/ mL), weak (>100–1,000 μg/mL), and inactive (>1,000 μg/mL); and synergism using Chou-Talalay criteria, namely additive effect (CI=1), synergistic (C<1), and antagonist (C>1).[12]

This research consists of 2 stages. Phase I is intended to determine the anticancer effect of SLAE compounds on T47D and MCF-7 breast cancer cell cultures. Stage 2 determines the co-chemotherapy effect of SLAE with doxorubicin

(DOX) on T47D and MCF-7 breast cancer cell culture.

The Health Research Ethics Committee of the Universitas Islam Bandung Faculty of Medicine has approved this study with a health research approval number: 112/Komite Etik.FK/III/2018.

Results

This study presented the cytotoxic test results of SLAE compound against T47D and MCF-7 breast cancer cell cultures. The MTT method produced IC_{50} values of SLAE in T47D cell culture of 86,029 µg/mL, IC_{50} MCF-7 166.5 µg/mL, DOX at T47D 10.3 µg/mL, while IC^{50} DOX at MCF-7 was 26.8 µg/mL. The average IC^{50} values and the standard deviations of SLAE and DOX can be seen in Figure 1.

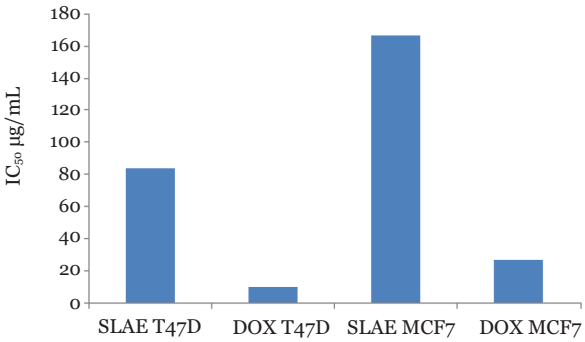

Figure 1 IC_{50} of Soursop Leaf Aqueous Extracts and Doxorubicin

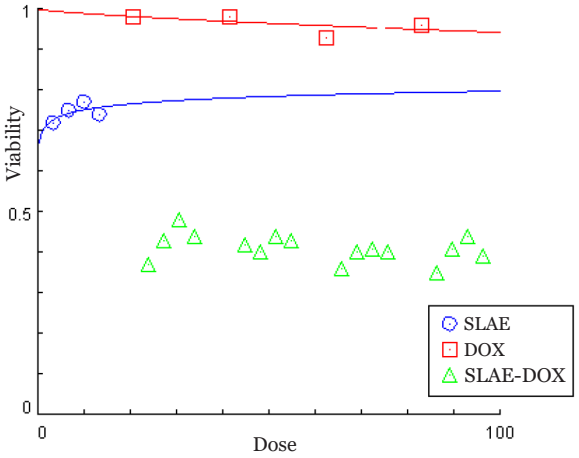

Figure 2 Effect Curve of SLAE, DOX, and SLAE-DOX Combination on MCF7

The results of a combination test of SLAE with DOX on MCF7 are presented in Table 1 and Figure 2. The combination index of SLAE compound and DOX on T47D is shown in Table 2 and Figure 3. Combination Index values indicate that the SLAE compound is antagonistic to DOX on MCF7 and T47D.

Discussion

In developing new anti-cancer drugs as candidates for cancer therapy agents, preclinical testing is crucial to understanding the potential

Table 1 Combination Index of SLAE Compound and DOX on MCF7

SLAE Concentration (µg/mL)	DOX Concentration (µg/mL)	Viability (%)	CI
13.4	62.44	0.4	2.3
13.4	41.63	0.44	2.39
0.44	20.80	0.47	1.67
10.05	83.25	0.41	1.2
10.05	62.44	0.41	3.6
10.05	41.63	0.48	1.2
10.05	20.80	0.41	3.1
6.7	83.25	0.41	2.44
6.7	62.44	0.4	3.5
6.7	41.63	0.4	3.5
6.7	20.80	0.43	1.19
3.35	83.25	0.35	1.12
3.35	62.44	0.36	7.67
3.35	41.63	0.42	8.53
3.35	20.80	0.37	5.27

Table 2 Combination Index of SLAE Compound and DOX on T47D

SLAE Concentration (µg/mL)	DOX Concentration (µg/mL)	Viability (%)	CI
5.15	43.01	0.33	2.3
5.15	32.26	0.39	7.2
5.15	21.5	0.36	12.74
5.15	10.75	0.35	15.46
3.86	43.01	0.31	26.1
3.86	32.26	0.36	9.5
3.86	21.5	0.38	6.5
3.86	10.75	0.39	5.4
2.57	43.01	0.35	7.7
2.57	32.26	0.33	11.5
2.57	21.5	0.32	14.13
2.57	10.75	0.33	11.51
1.29	43.01	0.32	7.11
1.29	32.26	0.36	3.24
1.29	21.5	0.3	10.7
1.29	10.75	0.32	7.09

for cytotoxic activity. Cytotoxic tests are used as an initial screening to determine the effect of a natural substance in inhibiting tumor cell growth. A compound has anti-cancer properties if it can hinder the development of 50% of tumor cell populations at specific concentrations. The requirement met for the cytotoxicity test system is that the test system must produce a reproducible dose-response curve with low variability. The response criteria must show a linear relationship with the number of cells, and the information obtained from the dose-response curve must be in line with the effect that appears. One method commonly used to determine cell numbers is the MTT.[16,17]

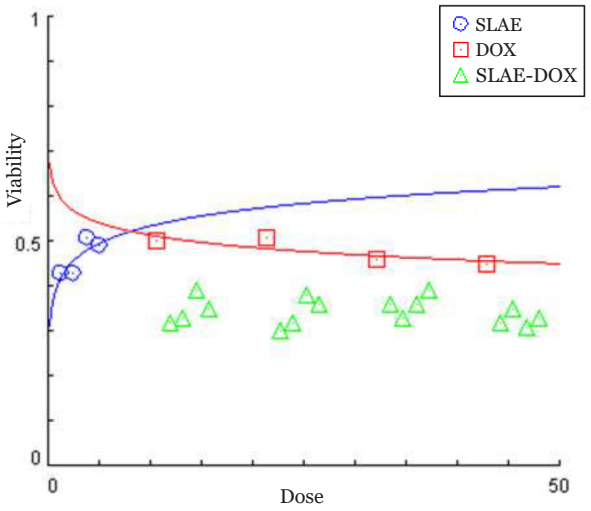

Figure 3 Effect Curve of SLAE, DOX, and SLAE-DOX Combination on T47D

The MTT method showed IC_{50} values of SLAE in T47D cell culture of 86.029 g/mL, and IC_{50} MCF-7 166.5 g/mL. This indicates weak cytotoxic activity against breast cancer cells.[15,18] It is similar to the study by Rady et al.,[19] which stated that soursop leaves have a cytotoxic effect on the liver (HepG2), breast (MCF-7), cervical (HeLa) cancer cells, and others. This cytotoxic effect occurs because of one of the bioactive components. Soursop is annonaceous acetogenins (AGE). Several purified AGEs showed cytotoxicity against various cancer cells, such as annonacin A or B showed cytotoxicity against HepG2; annomuricin A, B, or C against MCF-7 breast cells; annomuricin A, B, C or E against colonic cells HT-29 and pancreatic cells MIA Paca.[19,20]

Soursop leaves also trigger cell death through various mechanisms. Research shows increased apoptosis in MB-468 breast cancer cells through caspase three activation. In HT-29 colorectal cancer cells, leaf extract induces apoptosis through the accumulation of reactive oxygen species (ROS) followed by mitochondrial membrane potential (MMP) destruction and caspase activation. It also upregulated Bax and downregulated BCL-2.[19]

The problems faced in the application of

chemotherapy are chemotherapeutic agents that are toxic to healthy tissue, decreased immune system, and drug resistance occurs; for this problem, the application of co-chemotherapy or combination therapy is needed. Combination therapy can increase the effectiveness of anticancer agents, use lower anticancer doses, decrease toxicity to healthy tissue, slow down and inhibit drug resistance, and allow synergy efficacy in cancer cells. In determining whether soursop leaf aqueous extract can be a co-chemotherapy with doxorubicin and cisplatin, a potential combination test with isobologram.[10,12]

We did a combination test of doxorubicin and soursop leaf water extract in this study. The results of the combination index above 1 indicated that the soursop leaf extract and doxorubicin combination was antagonistic. These results are different from previous studies, which showed the synergistic effect of doxorubicin with soursop on 4T1 breast cancer cell culture accompanied by a decrease in ROS yields. It shows the potential of soursop as chemotherapy with antioxidant effects.[21] The difference may result from different tumor types or can also be caused by both drugs acting on the same receptor, resulting in competitive inhibitors.[22,23] This study has research limitations because it was only carried out on two cell line cultures and only used one soursop leaf preparation.

Conclusions

The conclusion of this study are soursop leaf aqueous extract has weak anticancer properties against MCF7 and T47D breast cancer cell culture and a combination of soursop leaf aqueous extract compounds and doxorubicin have an antagonistic effect against MCF7 and T47D breast cancer cells.

Conflict of Interest

The authors have no conflict of interest to declare.

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