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Solanum nigrum Ethanolic Extract (SNE) Increases Cytotoxic Activity of Doxorubicin on MCF-7 Cell

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Abstract

Leunca (*Solanum nigrum* L.) is a potential source of natural anticancer agents. *Solanum nigrum* L. ethanolic extract (SNE) has cytotoxic activity in several cancer cell lines. We aimed to evaluate the ability of SNE to increase MCF-7 cell sensitivity to doxorubicin as a chemotherapeutic agent for breast cancer. Cell viability of SNE and its combination treatment with doxorubicin were conducted by 3-(4,5-dimethylthiazol-2-yl)-2.5-diphenyltetrazolium bromide (MTT) assay, and apoptosis assay was analyzed by Ethidium bromide-acridine orange method. The SNE showed a cytotoxic effect in the MCF-7 cell line with IC_{50} 50 µg/mL. Combination treated DOX-SNE resulted in a combination index (CI) value of 0.21, indicating strong synergism SNE and doxorubicin. The SNE 25 µg/mL combined with doxorubicin 100 nM optimally induced apoptosis of MCF-7 cells. We concluded that SNE is the potential to be developed as a co-chemotherapeutic agent through apoptosis induction though the molecular mechanism need to explore.

Keywords: *Solanum nigrum L. herb ethanolic extract, doxorubicin, MCF-7, apoptosis.*

INTRODUCTION

Abnormal cell proliferation leads the cancer cells. Cancer cells grow, divide uncontrolled, invade, and spread throughout the body (Cooper and Hausman, 2020). Breast cancer is the most common cancer-causing mortality in women and is the most common cancer in the world (Heer, *et al*., 2020). Overexpression of receptor estrogen, progesterone receptor, and human epidermal growth factor receptor 2 (HER2) are biomarker for breast cancer prognosis (Ulaner, *et al*., 2016). Hormonal therapy such as hormone replacement therapy (HRT) for postmenopausal, ovulation-stimulating medications, contraceptive pills increase the risk factor of developing breast cancer. Otherwise, the lifestyles such as alcohol consumption, diet, and smoking improve breast cancer risk (Momenimovahed and Salehiniya, 2019).

Therapy for breast cancer depends on the stage/stadium of the pathology and development of cancer cells. The main goals of therapy breast cancer are inhibiting cell proliferation or decreasing cancer cells' metastatic incidence, and prolonging patient life. Chemotherapy agents such

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as tamoxifen, anthracycline, 5-Fluorouracil are recommended and common in non- and metastatic breast cancer types (Wang, *et al*., 2018; Waks and Winer, 2019; Jouybari, *et al*., 2019). Doxorubicin (DOX) has been used for decades and has been modified to increase therapy effectiveness (Lankelma, *et al*., 1999; Franco and Ait-Oudhia, 2018; Ansari, *et al*., 2017). However, using high concentrations of DOX or prolonged time will cause drug resistance. So, to prevent the resistance of DOX is increasing the ability and effectiveness of doxorubicin. Based on several researches, combination with natural compound that has cytotoxic activity in cancer cells effectively improves the potency of DOX.

Leunca (*Solanum nigrum* L.) is a Solanaceae family. The active compounds of *S. nigrum* are glycoalkaloids, polyphenolic, polysaccharide, and glycoprotein, solanine, solasodine, and solamargine. Based on previous research, *Solanum nigrum* L. has cytotoxic activity on cancer cells by inducing apoptosis, G2/M cell cycle arrest, and autophagy (Elbehairi, *et al* 2020; Butt, *et al*., 2018; Ling, *et al*., 2019; Shirkavad, *et al*., 2019). The combination treatment of Leunca with chemotherapy agent increases the chemotherapy's sensitivity *in vitro* and reduces the toxicity *in vivo* (Sarmoko, *et al*., 2011; Maruti, *et al*.,2011; Rumiyati, *et al*., 2015). Co-chemotherapy increased the chemotherapeutic efficacy and reduced the resistance evidence on chemotherapy, allowing chemotherapy on the lower dosage of a chemotherapy agent that use for therapy.

This research aimed to investigate the potency of *Solanum nigrum* L. ethanolic extract (SNE) on MCF-7 breast cancer cells. The effectiveness of the combination of chemotherapy agents with SNE was observed to investigate the activity of SNE through increasing the cytotoxicity of DOX on MCF-7 cells, allowing the use of lower concentrations of SNE and DOX. The possible mechanism on the toxicity of SNE and DOX were observed through apoptosis evidence.

MATERIALS AND METHODS

Sample Preparation

Dried powder of *Solanum nigrum* L. obtained from Balai Besar Penelitian dan Pengembangan Tanaman Obat dan Obat Tradisional (B2P-2TOOT), Indonesia. The dried powder of *Solanum nigrum* L. was extracted in 95% ethanol for 7 days. The SNE was concentrated under a vacuum rotary evaporator.

Cells Lines

MCF-7 cell lines were provided by Prof. Tatsuo Takeya (Nara Institute of Science and Technology, Nara, Japan). The cells were cultured in complete medium culture consist of Dulbecco's Modified Eagle Medium (DMEM) (Gibco, New York, USA), 10%v/v Fetal Bovine Serum (FBS) (Gibco), and 1% Penicillin- Streptomycin (Gibco) at the temperature of 37°C and with a flow of 5% CO_2.

Drugs

Doxorubicin (DOX) (Ebewe vial 10 mg/5 mL) purchased from PT. Ferron Par Pharmaceutical (Cikarang, Indonesia) was diluted directly in a culture medium.

Single and Combination Cytotoxic Assay

The cytotoxic assay was measured using MTT method. MCF-7 cells were adjusted to $5x10^3$ cells/well and cultured in 100 µL culture medium in 96 well plates and then incubated one day. The cells were treated with various concentrations of SNE (1-80 µg/mL) and various concentrations of DOX (0.25-0.8 µM). After 24 h incubation, MTT reagent was applied, followed by 4 h incubation. 10% v/v SDS in 0.1N HCl as stopper reagent was then applied. The plate was then kept with protection from light overnight, continued with absorbance determination (λ 595 nm) using ELISA reader (Bio-Rad, Hercules, California, USA). The absor-

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bance was used to calculate IC_{50} value, which is the concentration inhibiting 50% of cell growth. To investigate whether SNE increases the sensitivity of DOX, SNE was treated in combination with DOX. Combinational treatment was evaluated by calculating Combination Index (CI) value (Reynolds and Maurer, 2005):

$$CI=D_1/D_{x1} + D_2/D_{x2}$$

D_1 and D_2 represent concentrations used in combinational treatment, while D_{x1} and D_{x2} are single treatment concentration giving the same response as D_1 and D_2, respectively. CI value represents the potency of SNE in combination treatment with DOX.

Statistic

All experiments were independently repeated at least three times. Student's t-test determined statistical significance for single and combination cytotoxicity.

Apoptosis Assay

The $5x10^3$ cells were platted on coverslip and treated with SNE and DOX for 15 h. At the end of incubation, cells were washed PBS and were added 10 µL of ethidium bromide-acridine orange

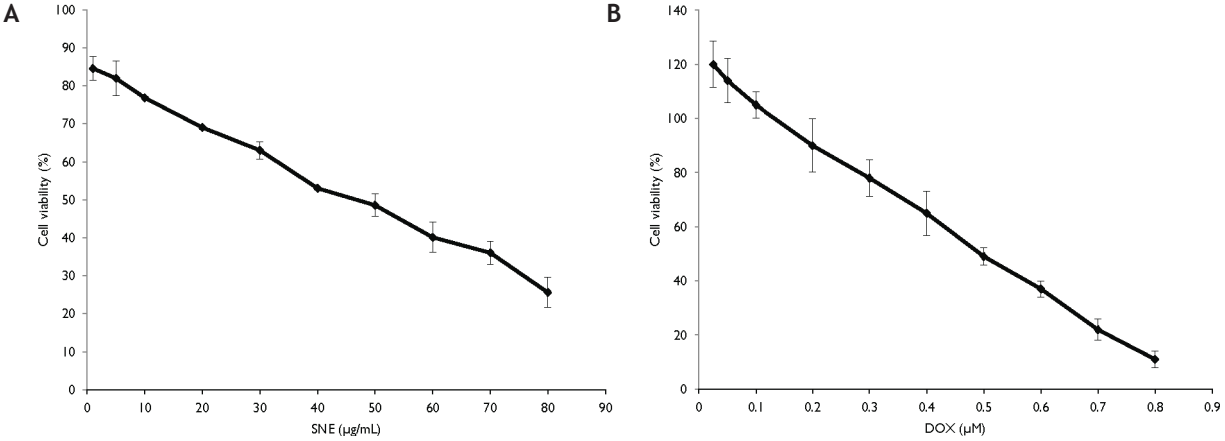

Figure 1. Single Cytotoxic Effect of SNE and DOX on MCF-7 Cells. (A) Treatment SNE (1-80 µg/mL) on MCF-7 and (B) treatment DOX (0.25-0.8 µM) on MCF-7 cell for 24 h was measured using MTT assay. Data are represented as mean -/+ SE.

reagent (Sigma, USA) 5 µg/mL each coverslip. Cells were observed under a fluorescent microscope. Viable cells had green fluorescence, and apoptotic cells (early and late0.) had orange fluorescence with showing apoptotic bodies.

RESULTS

Cytotoxic Effect of SNE and DOX on MCF-7 Cells

Firstly, we investigate SNE and DOX's cytotoxic activity on MCF-7 breast cancer cells by MTT assay. Single cytotoxicity assay of SNE was done in various concentrations on MCF-7 cells. SNE showed a cytotoxic effect in a dose-dependent manner with IC_{50} value of SNE was 50 µg/mL (Figure 1A). The results suggest that SNE induced toxicity on breast cancer cells.

Synergistic Effect of Combination Treatment SNE and DOX on MCF-7 Cells

Next, we generated the ability of SNE in combination with the chemotherapy agent, DOX. Based on previous research, DOX showed a cyto-

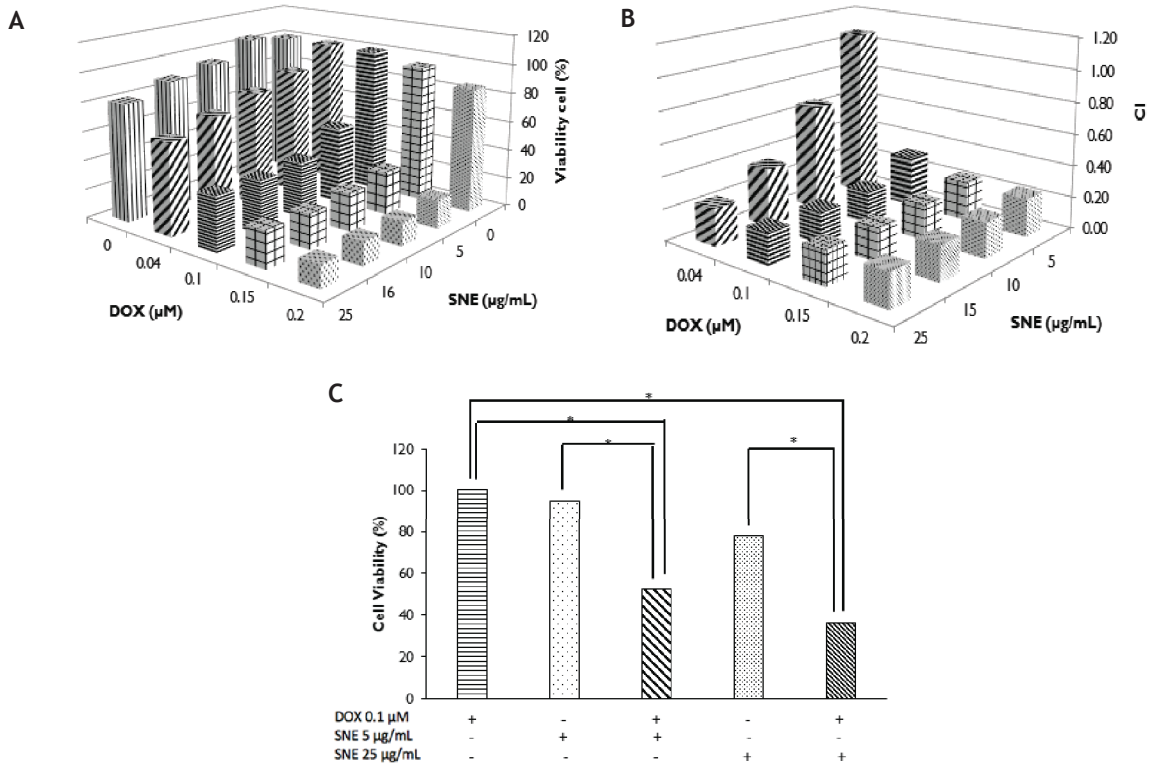

Figure 2. Combination Cytotoxic Effect of SNE and DOX on MCF-7 Cells. (A) The viability cells of combination treatment SNE with DOX on MCF-7 for 24 h was measured using MTT assay and (B) The CI value of combination treatment SNE-DOX. (C) The viability cells of SNE (5 and 25 µg/mL) with DOX (0.1 µM). Data are represented as mean -/+ SE. $p<0.05$ (Student's t-test).

toxic effect with IC_{50} 400 nM or 0.4 µM. We investigated the cytotoxic of DOX and showed with IC_{50} 0.5 µM (Figure 1B). We therefore investigate the combination effect of SNE with DOX. The results showed that combination SNE and DOX with several doses (under IC_{50}). Treatment of SNE and DOX on MCF-7 cells was conducted to investigate the ability of SNE to increase DOX's cytotoxicity on MCF-7 cells. The combination of SNE and DOX decreased cell viability rather than DOX solely (Figure 2, Tabel 1). The CI was calculated to evaluate the synergistic effect of the combination. According to the CI values as described by Reynolds and Maurer (2005), all of the tested combinations gave a synergistic effect, and some of them are strong synergist (Table 2). This result indicates that a combination of SNE and Dox is a more effective treatment than a single DOX treatment.

Combination SNE and DOX Induce Apoptosis Incidence of MCF-7 Cells

The synergetic effect combination of SNE and DOX showed in decreasing of viability cells. To understand the possible mechanism, we investigated the apoptosis incidence in combination treatment SNE and DOX. The Determination of apoptosis was carried under microscope fluorescence. The acridine orange permeates into nucleus and produces the green color. The Ethidium Bromide was taken up by the cell with losing the integrity of cytoplasmic membrane. SNE and DOX treatment showed apoptosis, and combination treatment increasing apoptosis events (Figure 3). The result suggest that SNE possibilities to increase the potency of apoptosis cells combined with DOX on breast cancer cells though the quantity of apoptotic event should be done.

Tabel 1. Cell viability of combination treated, DOX and SNE.

Cell viability (%)		DOX (μM)				
		0	**0.04**	**0.1**	**0.15**	**0.2**
	0	100.00	100.25	99.76	94.35	85.20
SNE	**5**	94.92	86.43	52.30	29.44	19.05
(μg/mL)	**10**	93.41	78.73	37.54	25.40	16.11
	16	88.25	70.71	35.95	23.02	16.03
	25	78.13	62.78	36.51	24.84	14.76

DISCUSSION

Cytotoxic effect of leunca aginst cancer cell has been reported before. Leunca (*Solanum nigrum* L.) has been known to have cytotoxic effect against on several cancer cells (Sarmoko, *et al*., 2011; Maruti, *et al*., 2011; Moglad, 2018; Churiyah, *et al*., 2020). SNE was found to have low genotoxicity activity and acute toxicity on mice (Rumiyati, *et al*., 2015). This study investigated the cytotoxic of SNE on MCF-7 cells (Figure 1), with IC_{50} 50 μg/mL. The SNE showed high toxicity on MCF-7 cells compared to HeLa cervical cancer cells and WiDr colon cancer cells. This finding indicated that SNE had cytotoxic potency towards MCF-7 cells.

We investigated the cytotoxic of DOX and used it for reference of combination with SNE. Based on previous research, a combination of leunca with several chemotherapy agents has been known to increases the cytotoxic of chemotherapy agents in several cancer cells (Sarmoko, *et al*., 2011; Istiaji, *et al*., 2010; Maruti, *et al*., 2011; Anindyajati, *et al*., 2010). The combination of SNE with DOX increased the cytotoxicity of DOX, reduced the cell viability and proliferation of MCF-7 cells. The combination of SNE and DOX showed synergistic mechanism. The lowest concentration of SNE-DOX showed antagonism mechanism. The synergistic effect of SNE-DOX showed consistency with the results in the amount of cell viability. This result suggests that combination SNE-DOX possibility to induce apoptosis on MCF-7 cells.

We investigate the apoptosis event of a combination of SNE and DOX used Immunofluorescence Ethidium Bromide-Acridine orange assay. The apoptosis showed an orange color in cells and fragmentation body of cells. The single treatment of SNE and DOX showed inducing apoptosis, and interestingly, the combination of SNE-DOX more induces the evidence of apoptosis (Figure 3) although molecular mechanism should to be investigate. The synergistic mechanism of the reducing cell viability on the combination of SNE-DOX possibility induce apoptosis mechanism while the protein that responsibility the apoptosis should be explored. This result finds that the combination of SNE-DOX increases the sensitivity of DOX.

CONCLUSION

The SNE showed cytotoxic activity on MCF-7 breast cancer cells and induces the sensitivity the cells upon DOX treatment. The combina-

Tabel 2. Combination Index value of combination treatment DOX and SNE.

CI		DOX concentration (μM)			
		0.04	**0.1**	**0.15**	**0.2**
SNE (μg/mL)	**5**	1.07	0.31	0.24	0.24
	10	0.67	0.20	0.21	0.22
	15	0.36	0.19	0.20	0.22
	25	0.23	0.20	0.21	0.21

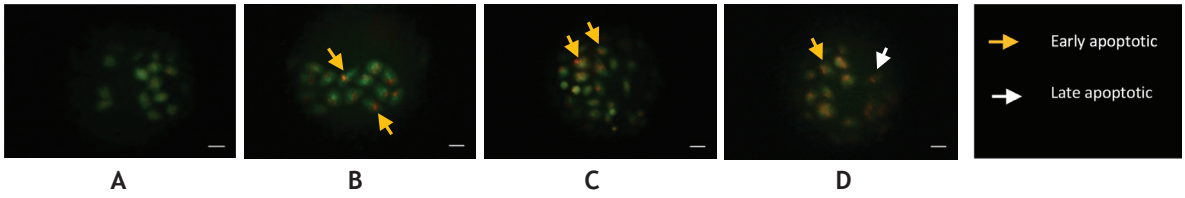

Figure3. Apoptosis of SNE and DOX on MCF-7 Cells. Cell treated with SNE and DOX for 15 h. (A) untreated, (B)SNE 25 µg/mL, (C) DOX 0.1µM, and (D). SNE 25 µg/mL-DOX 0.1µM. Scale bare 10 µm.

tion of SNE with chemotherapy agents decreases the usage concentration of DOX in this research, reduces the use of high concentration for doxorubicin as cancer therapy *in vitro* assay. So this data can be used to explore the prospect of SNE to reduce chemotherapy agent resistance evidence but it needs to explore more. The combination of SNE-DOX-induced the apoptosis mechanism and needs to be explored further about the protein of apoptosis that responsible for inducing apoptosis.

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