Global Medical and Health Communication

Online submission: https://ejournal.unisba.ac.id/index.php/gmhc
DOI: https://doi.org/10.29313/gmhc.v11i3.10985

GMHC. 2023;11(3):195–199
pISSN 2301-9123 | eISSN 2460-5441

RESEARCH ARTICLE

Cytotoxicity of Combination Doxorubicin and *Garcinia picrorrhiza* Fruit Extract on Fibroblast Cell

Sri Utami,[1] Susi Endrini,[1] Lilian Batubara,[1] Nunung Ainur Rahmah,[1] Irfan Syarif,[1] Said Nafik,[2] Betharie Cendera Arrahmani,[3] Agung Novianto,[4] Hanna Sari Widya Kusuma,[4] Wahyu Widowati[5]

[1]Faculty of Medicine, Universitas YARSI, Central Jakarta, Indonesia, [2]Directorate General of Intellectual Property, Ministry of Law and Human Rights Republic of Indonesia, South Jakarta, Indonesia, [3]Risk and Regulatory Consulting Pharma and Life Sciences, PricewaterhouseCoopers (PwC) GmbH WPG, Frankfurt, Germany, [4]Biomolecular and Biomedical Research Center, Aretha Medika Utama, Bandung, Indonesia, [5]Faculty of Medicine, Universitas Kristen Maranatha, Bandung, Indonesia

Abstract

Combining chemotherapeutic agents such as doxorubicin with herbal products or other compounds that can enhance cytotoxicity without side effects is required. Thus, we aimed to observe the cytotoxicity of doxorubicin and *sesoot* (*Garcinia picrorrhiza*) fruit ethanolic extract (GpKar) on human fibroblast cells, BJ. This study used a post-test-only control randomized group design with n=3 and a number group of 5. The method used in this research is cell number, and viability was measured with (3-(4,5-dimethylthiazol-2-yl)-5-(3-carboxymethoxyphenyl)-2-(4-sulfophenyl)-2H-tetrazolium assay. Treatments consisted of a combination of doxorubicin (0.02 µg/ml) and GpKar of 66.47 µg/ml (DES1), 132.94 µg/ml (DES2) and 265.89 µg/ml (DES3). The data were analyzed using a one-way ANOVA and Duncan post hoc tests. DES3 showed the lowest viability among treatments (89.32%). DES1 and DES2 showed high viability (>90%), 97.93%, and 95.08%, respectively. Thus, the combination of doxorubicin (0.02 µg/ml) and GpKar (66.47 µg/ml) was considered safe for further use in the following assay. In summary, the combination of doxorubicin and GpKar showed high viability in normal fibroblast cells.

Keywords: Cytotoxic, doxorubicin, *Garcinia picrorrhiza*, human fibroblast, MTS assay

Introduction

Cancer is a disease indicated by loss of control in cycle cell regulation and homeostasis in multicellular organisms. The deregulation of growth genes causes cancer and is insensitive to anti-growth signals.[1] According to the World Health Organization, cancer deaths have risen from 1.2 million to 1.8 million and are now the leading causes of death.[2] The incidence is thought to elevate in 2030 to 26 million people and 17 million deaths due to the disease. It grows faster in poor and developing countries. Report on Result of National Basic Health Research (*Riskesdas*) reported in 2007 that the prevalence of cancer in Indonesia was 4.3 out of 1,000 people.[3] Breast cancer is the most common cancer among women in Indonesia, with an incidence of 26 cases per 100,000 women, followed by cervix cancer, which has an incidence of 16 cases per 100,000 women.

Breast cancer has been commonly treated with chemotherapeutic agents, doxorubicin, yet it generates adverse effects such as dizziness, vomiting, and cardiac arrhythmias.[4] Therefore, a combination with herbal products or other compounds that can enhance cytotoxicity without side effects is required. Several studies utilized a combination of garcinol and doxorubicin that showed better performance compared to the single compound.[5] The cytotoxic effects of paclitaxel against breast cancer are enhanced by garcinol. Combination therapy, with a low dose of Taxol and garcinol, is a promising therapeutic strategy for managing advanced or metastatic breast cancer to determine the underlying mechanisms of these effects in vivo.[6] The synergistic combination therapy is recommended because it is safer and has a higher response rate than monotherapy.

Plant extracts have been widely used in anticancer therapy due to their high toxicity. Thus, it is essential to observe their toxicity on normal cells. A recent study shows that *Moringa oleifera* leaf extract synergistically enhanced the cytotoxic effect of cisplatin on PANC-1 cells.[7] Previous studies showed that the phytochemical of *sesoot* (*Garcinia picrorrhiza* Miq.) bark exhibited an antimutagenic effect on standard

Received: 15 December 2023; Revised: 30 August 2023; Accepted: 8 December 2023; Published: 23 December 2023

Correspondence: Dr. Hj. Sri Utami, S.Si., M.Si., S.H. Faculty of Medicine, Universitas YARSI. Jln. Letjen Suprapto No. Kav. 13, Central Jakarta 10510, Special Capital Region of Jakarta, Indonesia. E-mail: uutsuyono@yahoo.com

mutants, which has the potential to be used as an anticancer.[8] Combination of mandarin orange (*Citrus reticulata*) pericarp ethanolic extract-doxorubicin showed antiproliferative effects on Michigan Cancer Foundation-7 (MCF-7).[9] Combination of pyrophen fraction of endophyte fungus-doxorubicin showed a synergistic effect in antiproliferative, cell cycle, and apoptotic induction properties MCF-7.[10]

However, it remains unclear the effect of *Garcinia picrorrhiza* fruit ethanolic extract (GpKar) on normal cells except on Vero cells. The combination of doxorubicin and GpKar is expected to be toxic to cancer cells. Thus, we aimed to observe the cytotoxicity of GpKar and doxorubicin on human fibroblast cells, BJ.

Methods

Garcinia picrorrhiza Miq. fruit were obtained from Kebun Raya Bogor, LIPI. *Garcinia picrorrhiza* fruit is shown by the color of its seed, which is brown. The *sesoot*-ripe fruit was extracted using a maceration technique with 70% distillate ethanol for 24 hours and then filtered. The filtration was repeated until the colorless filtrate was further evaporated. The extract was stored at a temperature of −20°C.[11–13]

Biomolecular and Biomedical Research Center, Aretha Medika Utama, provided BJ cells [ATCC®CRL-2522]. Cells were grown in α-minimum essential medium Eagle (α-MEM) [Biowest L0475], 10% fetal bovine serum (FBS) [Biowest S181H], 1% penicillin-streptomycin [Biowest L0022], and maintained at 37°C in a humidified atmosphere and 5% CO_2 until the cells were 80–90% confluence. The growth medium was removed and washed with phosphate buffer saline (PBS) [Gibco 14200075]. Cells were added with trypsin-EDTA [Biowest L0931-500] and incubated at 37°C for 3 min. Tripsynization was stopped by adding a growth medium in equal volume. Cells were suspended and replaced into a tube, centrifuged at 500 xg for 4 min. The supernatant was removed, and the pellets were resuspended with 4–5 ml growth medium. The cell suspension was aliquoted into a T-flask containing a growth medium with a density of 8,000 cells/cm^2. Medium was replaced every two days. Cells were incubated at 37°C, 5% CO_2.[14]

This experimental study used a post-test-only control randomized group design with n=3, number group of 5. The treatment group consisted of negative control (normal cells), DMSO control, DSE 1 (doxorubicin of 0.02 µg/ml + GpKar of 66.47 µg/ml), DSE 2 (doxorubicin of 0.02 µg/ml + GpKar of 132.94 µg/ml), and DSE 3 (doxorubicin of 0.02 µg/ml + GpKar of 265.89 µg/ml).

The cells number and viability were measured with 20 µl (3-(4,5-dimethylthiazol-2-yl)-5-(3 carboxymethoxyphenyl)-2-(4-sulfophenyl)-2H-tetrazolium (MTS) [Promega, Madison, WI, USA], and incubated at 37°C for 3 hours. Briefly, 100 µl cells were plated (5×103 cells per well) and incubated for 24 h at 37°C in a humidified atmosphere and 5% CO_2. The medium was then discarded and added with 90 µl of new medium and 10 µl of a combination of doxorubicin (0.02 µg/ml) and GpKar (66.47, 132.94, and 265.89 µg/ml) in DMSO in different plates in triplicate then incubated for 24 h. Untreated cells served as the control. The 20 µl MTS was added to each well. The plate was incubated in 5% CO_2 at 37°C incubator for 4 hours. The absorbance was measured at 490 nm on a microplate reader [MultiSkan Go Thermoscientific]. The data is presented as the percentage of viable cells (%). The viability assay determined the safe and non-toxic concentration for the following assay.[7,15]

Results

As shown in Figure, the combination of doxorubicin and *sesoot* extract was toxic to BJ cells compared to the control. Combining doxorubicin 0.02 µg/ml and an extract of 265.89 µg/ml showed the lowest viability among treatments (89.32%). Extracts of 66.47 µg/ml (DES1) and 132.94 µg/ml (DES2) showed high viability (>90%), which were 97.93% and 95.08%, respectively. However, DES1 was the only treatment comparable to the control, while DES2 showed a significant difference compared to the control. Thus, DES1 was considered safe for further use in the following assay.

Discussion

The present study showed that a combination of doxorubicin and *Garcinia picrorrhiza* extract showed high viability on normal fibroblast cells. This study used *Garcinia picrorrhiza* ethanolic extract and MTS assay for measuring cytotoxicity in human fibroblast cells, which are a novelty in experimental research. However, the limitation of this study is there need to be a characterization of the compounds in *Garcinia picrorrhiza*.

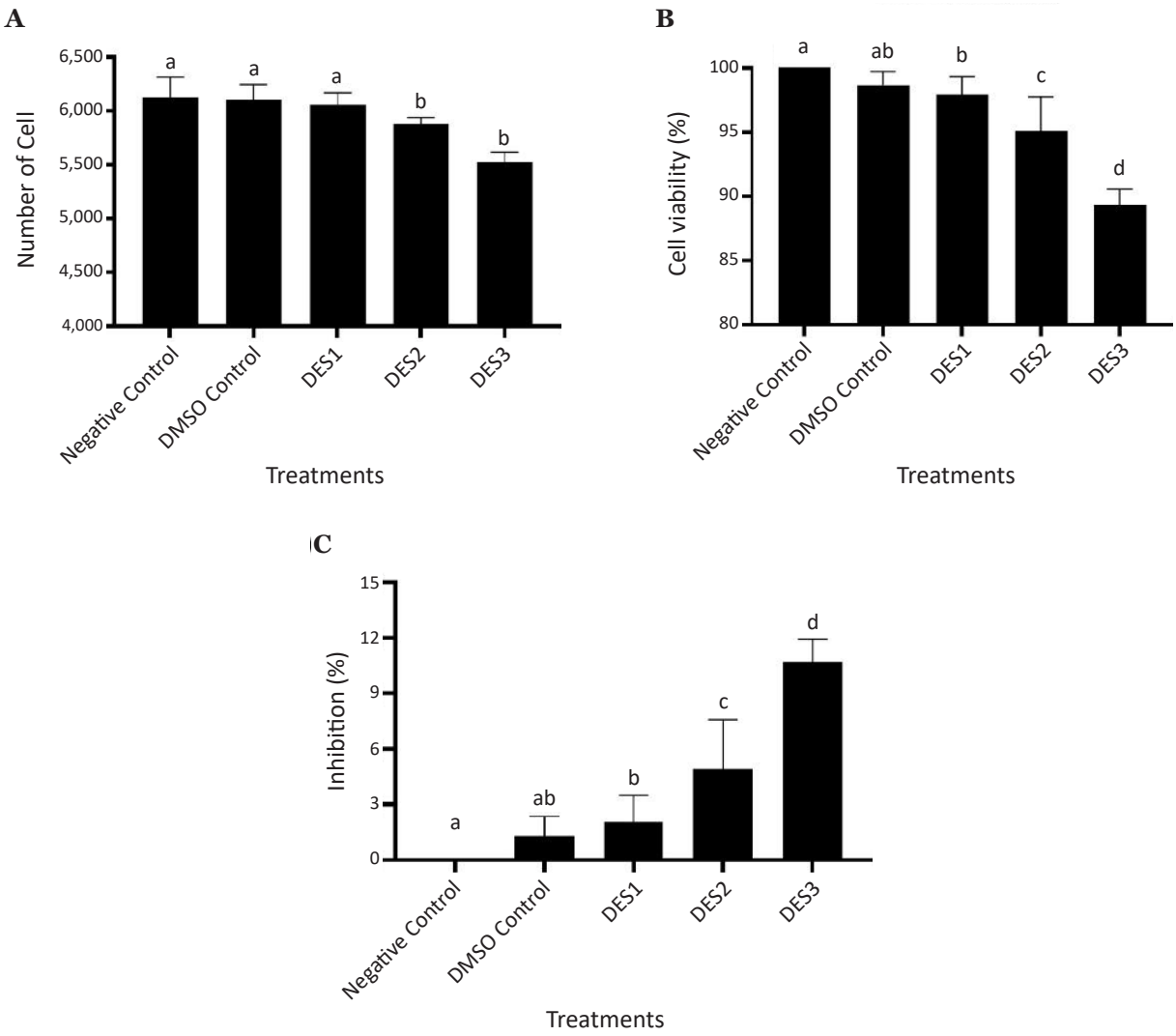

Figure Fibroblast Cells Treated with GpKar

Note: (**A**) Number of cell. (**B**) Cell viability (%). (**C**) Inhibition cell (%). DES1: doxorubicin 0.02 µg/ml and GpKar 66.47 µg/ml. DES2: doxorubicin 0.02 µg/ml and GpKar 132.94 µg/ml. DES3: doxorubicin 0.02 µg/ml and GpKar 265.89 µg/ml. The data is shown as means±SD with n=3. Different letters (a, ab, b, c, d) show significant differences among GTE doses based on the Duncan post hoc test (SPSS version 20.0) with p=0.05

Based on another study, the measurement of cell cytotoxicity of *Garcinia mangostana* used an MTT assay. Tannin compounds at 12.5%, 25%, 50%, and 100% in *Garcinia mangostana* extract show higher proliferation ability compared to the concentration of 6.25%. Tannins from mangosteen peel extract showed slightly toxic properties at a concentration of 6.25% and non-toxic at concentrations less than 6.25%, equivalent to 2.20% tannin isolation from mangosteen peel extract.[16]

Several studies utilized the combination of sulbactam and doxorubicin to enhance the cytotoxicity of doxorubicin in breast cancer cells.[17] These results indicate that combining both compounds can be used in cancer therapy without affecting normal cells. Therefore, previous studies utilized a combination of garcinol and doxorubicin that showed better performance compared to the compound alone.[19] Garcinol or Cambogia, a benzophenone derivative, can be obtained from *Garcinia indica* and *Garcinia picrorrhiza* Miq.[19] Furthermore, the higher concentration showed the lower cell proliferation rate described, namely the cytotoxic effect of flavin and EGCG on RL-34 cells, which states that all polyphenols will produce cytotoxicity at high concentrations.[20] Based on the results of this

study, tannins of *Garcinia mangostana* Linn. peel extract 0.78% compared with chlorhexidine gluconate 0.2% had better biocompatibility in BHK-21 fibroblast cells.[21]

Phytochemical compounds of Garcinia have been studied and showed the presence of prenylated xanthones, bioflavonoids, and benzophenones.[22] Garcinol on MCF-7, which has a positive estrogen receptor, and MDA-MB 231 with the negative receptor, showed growth inhibition and induced apoptosis on cancer-specific cells. Thus, garcinol alone can benefit as a chemopreventive agent, mainly in breast cancer.[23,24]

Conclusions

In conclusion, the combination of doxorubicin and GpKar showed high viability in normal fibroblast cells. These results indicate that combining both compounds can be used in cancer therapy without affecting normal cells.

Conflict of Interest

The authors declared that they have no competing interests.

Acknowledgments

We gratefully acknowledge the financial and facilities support provided by Universitas YARSI research grant 2016 and Aretha Medika Utama Biomolecular and Biomedical Research Center, Bandung, Indonesia. We thank Fadhilah Haifa Zahiroh, Adilah Hafizha Nur Sabrina, Faradhina Salfa Nindya, Nindia Salsabila Mia Dewi, Vini Ayuni, and Annisa Firdaus Sutendi from Aretha Medika Utama, Biomolecular and Biomedical Research Center for their technical support.

References

1. Baylin SB, Jones PA. Epigenetic determinants of cancer. Cold Spring Harb Perspect Biol. 2016;8(9):a019505.
2. World Health Organization. The top 10 causes of death [Internet]. Geneva; World Health Organization; 2020 [cited 2022 September 12]. Available from: https://www.who.int/news-room/fact-sheets/detail/the-top-10-causes-of-death.
3. National Institute of Health Research and Development, Ministry of Health, Republic of Indonesia. Report on result of national basic health research (Riskesdas) 2007 [Internet]. Jakarta: National Institute of Health Research and Development, Ministry of Health, Republic of Indonesia; 2008 [cited 2022 September 15]. Available from: https://repository.badankebijakan.kemkes.go.id/id/eprint/4386/1/Report%20on%20Result%20of%20National%20Basic%20Health%20Research%202007.pdf.
4. Tamargo J, Caballero R, Delpón E. Cancer chemotherapy and cardiac arrhythmias: a review. Drug Saf. 2015;38(2):129–52.
5. Zhang Y, Li H, Zhang J, Zhao C, Lu S, Qiao J, et al. The combinatory effects of natural products and chemotherapy drugs and their mechanisms in breast cancer treatment. Phytochem Rev. 2020;19(5):1179–97.
6. Tu SH, Chiou YS, Kalyanam N, Ho CT, Chen LC, Pan MH. Garcinol sensitizes breast cancer cells to Taxol through the suppression of caspase-3/iPLA$_2$ and NF-κB/Twist1 signaling pathways in a mouse 4T1 breast tumor model. Food Funct. 2017;8(3):1067–79.
7. Hidayat M, Soeng S, Prahastuti S, Erawijantari PP, Widowati W. Inhibitory potential of ethanol extract of detam 1 soybean (*Glycine max*) seed and jati belanda (*Guazuma ulmifolia*) leaves on adipogenesis and obesity models in 3T3-L1 cell line. JSRR. 2015;6(4):304–12.
8. Sukandar ER, Kaennakam S, Raab P, Nöst X, Rassamee K, Bauer R, et al. Cytotoxic and anti-inflammatory activities of dihydroisocoumarin and xanthone derivatives from *Garcinia picrorhiza*. Molecules. 2021;26(21):6626.
9. Adina AB, Goenadi FA, Handoko FF, Nawangsari DA, Hermawan A, Jenie RI, et al. Combination of ethanolic extract of *Citrus aurantifolia* peels with doxorubicin modulate cell cycle and increase apoptosis induction on MCF-7 cells. Iran J Pharm Res. 2014;13(3):919–26.
10. Astuti P, Januarti IB, Kiromah NZ, Fitri HA, Wahyono W, Wahyuono S. Pyrophen isolated from the endophytic fungus *Aspergillus fumigatus* strain KARSV04 synergizes the effect of doxorubicin in killing MCF7 but not T47D cells. Turk J Pharm Sci. 2020;17(3):280–4.

11. Widowati W, Mozef T, Risdian C, Yellianty Y. Anticancer and free radical scavenging potency of *Catharanthus roseus*, *Dendrophthoe pentandra*, *Piper betle* and *Curcuma mangga* extracts in breast cancer cell lines. Oxid Antioxid Med Sci. 2013;2(2):37–42.
12. Widowati W, Fauziah N, Herdiman H, Afni M, Afifah E, Kusuma HS, et al. Antioxidant and antiaging assays of *Oryza sativa* extracts, vanillin, and coumaric acid. JNR. 2016;16(3):88–99.
13. Widowati W, Prahastuti S, Ekayanti NLW, Munshy UZ, Kusuma HSW, Wibowo SHB, et al. Anti-inflammation assay of black soybean extract and its compounds on lipopolysaccharide-induced RAW 264.7 cell. J Phys Conf Ser. 2019;1374:012052.
14. Girsang E, Lister INE, Ginting CN, Nasution SL, Suhartina S, Munshy UZ, et al. Antioxidant and anti-inflammatory activity of chlorogenic acid on lead-induced fibroblast cells. J Phys Conf Ser. 2019;1374:012006.
15. Widowati W, Wijaya L, Wargasetia TL, Bachtiar I, Yellianty Y, Laksmitawati DR. Antioxidant, anticancer, and apoptosis-inducing effects of Piper extracts in HeLa cells. J Exp Integr Med. 2013;3(3):225–30.
16. Hadi FP, Wahjuningrum DA, Cahyani F. Uji toksisitas tanin dari kulit manggis (*Garcinia mangostana* L.) terhadap sel fibroblas BHK-21. Conserv Dent J. 2015;5(1):6–11.
17. Wen SH, Su SC, Liou BH, Lin CH, Lee KR. Sulbactam-enhanced cytotoxicity of doxorubicin in breast cancer cells. Cancer Cell Int. 2018;18:128.
18. Sethi G, Chatterjee S, Rajendran P, Li F, Shanmugam MK, Wong KF, et al. Inhibition of STAT3 dimerization and acetylation by garcinol suppresses the growth of human hepatocellular carcinoma in vitro and in vivo. Mol Cancer. 2014;13:66.
19. Utami S, Sachrowardi QR, Damayanti NA, Wardhana A, Syarif I, Nafik S, et al. Antioxidants, anticollagenase and antielastase potentials of ethanolic extract of ripe soot (*Garcinia picrorrhiza* Miq.) fruit as antiaging. J Herbmed Pharmacol. 2018;7(2):88–93.
20. Feng Q, Torii Y, Uchida K, Nakamura Y, Hara Y, Osawa T. Black tea polyphenols, theaflavins, prevent cellular DNA damage by inhibiting oxidative stress and suppressing cytochrome P450 1A1 in cell cultures. J Agric Food Chem. 2002;50(1):213–20.
21. Werdiningsih M, Ira W, Cahyani F. Biocompatibility of 0.78% tannin of *Garcinia mangostana* Linn. pericarp extract and 0.2% chlorhexidine gluconate against BHK-21 fibroblast cells culture. Conserv Dent J. 2020;10(1):36–39.
22. Hassan NKNC, Taher M, Susanti D. Phytochemical constituents and pharmacological properties of *Garcinia xanthochymus*-a review. Biomed Pharmacother. 2018;106:1378–89.
23. Ye X, Yuan L, Zhang L, Zhao J, Zhang CM, Deng HY. Garcinol, an acetyltransferase inhibitor, suppresses proliferation of breast cancer cell line MCF-7 promoted by 17β-estradiol. Asian Pac J Cancer Prev. 2014;15(12):5001–7.
24. Choudhury B, Kandimalla R, Elancheran R, Bharali R, Kotoky J. *Garcinia morella* fruit, a promising source of antioxidant and anti-inflammatory agents induces breast cancer cell death via triggering apoptotic pathway. Biomed Pharmacother. 2018;103:562–73.