ANTICANCER ACTIVITY OF MANGOSTEEN PERICARP DRY EXTRACT AGAINST MCF-7 BREAST CANCER CELL LINE THROUGH ESTROGEN RECEPTOR -α

by Agustina Setiawati

Research Article

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Agustina Setiawati[1], Florentinus Octa Dika Riswanto[1], Sri Hartati Yuliani[1], Enade Perdana Istyastono[2,]

[1]Faculty of Pharmacy, Sanata Dharma University, Yogyakarta 55281, Indonesia

[2]Center for Environmental Studies Sanata Dharma University (CESSDU), Soropadan, Condongcatur, Yogyakarta 55283, Indonesia

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*Corresponding author
Agustina Setiawati

Email :
nina@usd.ac.id

ABSTRACT

Breast cancer has very complex morphological and molecular characteristic. Estrogen receptor is one of biomarker in breast cancer progression, more than 60% breast cancer overexpress estrogen receptor α (ERα). Xanthone in *Garcinia mangostana* was investigated whether to have anticancer activity on colorectal, prostate, lung, blood and breast cancer. This research was focused on molecular mechanism of anticancer activity of mangosteen pericarp extract (MPE) on ER-α. This study used MCF-7 cells as a model of ER-α overexpressed breast cancer cells. Cytotoxic study towards MCF-7 cells was designed by using MTT test, further apoptotic induction assay was determined by double staining method using acridine orange and ethidium bromide. Extract molecular mechanism against breast cancer was assayed by immunocytochemistry. The MTT data was analyze using probit analysis to get IC_{50} then apoptosis and immunocytochemistry data were analysis qualitative analysis. MPE had strong cytotoxic activity on MCF-7 cells with IC_{50} of 45µg/mL and morphological changes passed through apoptosis induction. The expression of ER-α in MPE treated cells was same as untreated cells. MPE did not suppress ER-α in both nucleus and cytoplasm. Anticancer activity of MPE misht be mediated by other gene involved in ER-α signaling pathway in breast cancer cells.

Key words: Breast cancer, *Garcinia mangostana* peel extract, estrogen receptor-α

INTRODUCTION

The most commonly diagnosed types of cancer among women in 2012 was breast cancer and breast cancer is expected to account for 29% (226,870) of all new cancer cases among women (Siegel *et al.*, 2012). In 2005, 186.467 women suffered from breast cancer and 41.116 of them passed away because of these disease (CDC, 2009). Breast cancer was the major caused death cancer after cervical cancer in Indonesia (Idamardi, 2007).

Breast cancer has very complex morphological and molecular characteristic. One important marker of breast cancer for prognosis is estrogen reseptor (Giacinti *et al.*, 2006). Approximately 60% of breast cancer over expressed estrogen receptor-α (ER-α) (Hanstein *et al.*, 2004). The development of molecular targeted drug has great advanced to cancer therapy. ER-α is a molecular target to stop the progression of breast cancer.

Mangosteen (*Garcinia mangostana* Linn) pericarp contains various phytochemicals, primary xanthones. Xanthon isolated from mangosteen pericarps were: α-mangostin, β-mangostin, γ-mangostin, mangostinone and 2-isoprenyl-1,7 dihydroxy-3-methoxy xanthone inhibited human leukimia HL60 cell line. Among them, α-mangostin supresed proliferation and induced apotosis of HL60 cells at 10µM (Matsumoto *et al.*, 2003). Xanthone extract (81% α-mangostin and γ mangostin) suppressed HT116 colon cancer cell (Aisha *et al.*, 2012; Nabandith *et al.*, 2004; Nakagawa *et al.*, 2007). Xanthone: α-mangostin, β-mangostin, γ-mangostin had anticancer activity on DL (Akao *et al.*, 2008). Alpha mangostin had antiproliferative activity against SKBR3 human breast adenocarcinoma cell line (Moongkarndi *et al.*, 2004) and MDA-MB231 (Kurose *et al.*, 2012). This compound were also proof to reduce lymph node

metastasis in xenograft model in Balb/c mice (Shibata *et al.*, 2011). *Garcinia mangostana* Linn pericarp extract has potential property to be chemopreventive agent against breast cancer.

This study investigated *Garcinia mangostana* pericarp extract (MPE) activity against breast cancer. Furthermore, it focused on ERα as molecular target to suppress proliferation of MCF-7 cell, in vivo model of ER-α over expressed breast cancer cell.

MATERIAL AND METHODS

MPE was purchased from PT. Borobudur had 28.10% α-mangosteen. Fructus cortex of *Garcinia mangostana* Linn was extracted use 70% ethanol with ratio botanical extract 1 and used maltodextrine as its excipient. MCF-7 breast cancer cell line was cultured in Parasitology laboratory, Faculty of Medicine, Gadjah Mada University. Apoptosis staining using ethidium bromide and acridine orange from SIGMA. This research used US BIO estrogen receptor α primary antibody.

MTT Cytotoxic Assay

MCF-7 cells breast cancer cell line were subcultured until confluent. Approximately 5×10^3 cells were seeded into 96-well microplate and incubated at 37°C and 5% CO_2 for 24h. Medium was fride treatment control, were prepared from Tamofen® tablet. MPE and tamoxifen were dissolved in DMSO as stock solutions. Various concentration of MPE and tamoxifen in medium were added into 96-well plate 100μL each and incubated in the same as previous condition for 24h. Each concentration was assayed in triplicates (n=3). The medium culture was removed and rinsed by PBS 10%, one hundreds ro Liter I 100μL contain 5mg/mL MTT was added into each well and incubated feather for 4h. Event, medium containing MTT was removed and 100μL SDS was added each well to dissolve the optical density of the resulting solution. The 96-well microplate was incubated for 24h and avoid from light contact. Formazan crystals were detected by ELISA reader using 595nm wavelength.

Apoptosis Assay

MCF-7 were seeded 5×10^4/100μL into coverslip in 24-well plate, then adapted in 37°C

and 5% CO_2 for 24h. Further, the medium was removed and rinsed by PBS. Seventeen and 34μg/mL of MPE in Dulbecco's Modified Eayle's Medium were added into the well plate and incubated for 24h. These concentrations were determined fariure calculation IC_{50}, as estimated concentration closed to IC_{50}. The medium was removed from MCF-7 cells and rinsed by PBS. The coverslip was removed from well plate and transferred to object glass, and acridine orange-ethidium bromide (AE) was dropped at the coverslip. This slide was soon observed under flouresence microscope.

Immunocytostaining of ER-α

MCF-7 cells in DMEM was seeded 10^5/100μL on 6-well plate and incubated under 5% CO2 for 24h. MPE in concentration of 17 and 34μg/mlL were applied on cells and incubated for 24 hours. After incubation, cells was collected and washed in PBS. They were resuspended in DMEM and placed in object glass, fixated for 5min, incubated in H_2O_2 for 10-15min and then washed again using PBS. Monoclonal antibody anti ER-α was and incubated at least for an hour and washed three time using PBS. Secondary antibody of biotinylated goat anti-polyvalent was added and incubated at room temperature for 10min and washed four times in PBS. DAB as chromogen the mistune was dropped and incubated for and 3-8min, washed using aquadest. Finally, hematoxylin solution was added and incubated for 3-4 minutes. This slides were dried and observed under light microscope. Expression of ER-α was showed by brown cell colour.

Analysis

The cytotoxic data of MPE and tamoxifen were analyzed by excel. IC_{50} was then calculated using probit analysis (Finney and Stevens, Any new references?)

RESULT AND DISCUSSION

This study investigated another cytotoxic propert of *Garcinia mangostana* pericarp against breast cancer cell line. MCF-7 was used as in vivo model of over expressed ER-α cells. MPE and tamoxifen we dissolved into excess DMSO as inert solvent in cell culture. Cavalli *et al.* (2011) and Licciardi *et al.* (2010) showed that DMSO 2μL in volume (2% v/v) have no
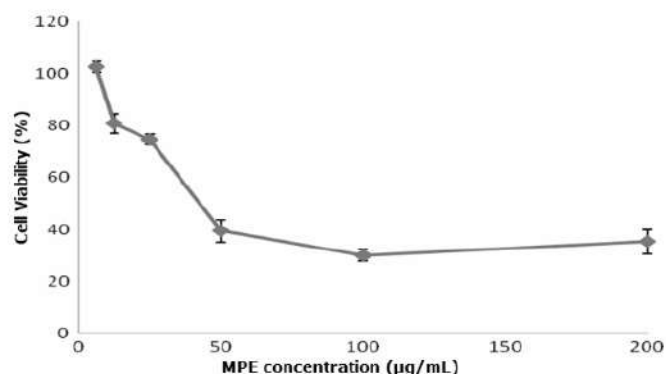
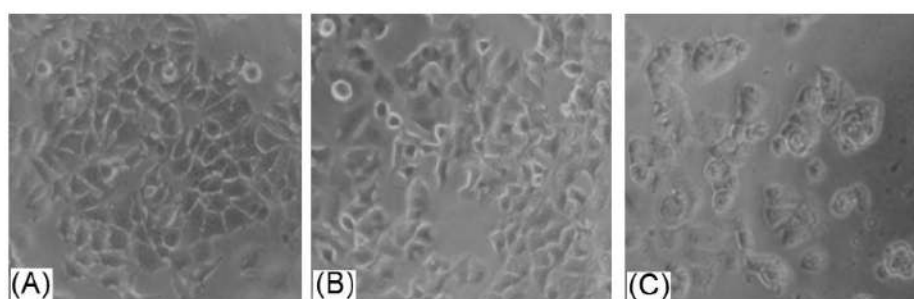Figure 1. Effect of MPE on MCF-7 viability after 24 hour incubation

Figure 2. Effect of MPE and tamoxifen on MCF-7 morphology after 24h incubation (A) Untreated cells (B) 40μg/mL MPE treated cells (C) 10-6μg/mL tamoxifen treated cells.

cytotoxic activity against MCF-7 cells. This research used 0.02% v/v DMSO for MPE and oxifen. MTT was absorbed into living cells and converted into purple coloured-formazan complex by succinate dehydrogenase in mitochondria (Doyle *and* Grifith, 2000). MPE exhibit cytotoxic parabolic profile (Figure 1), probit analysis yielded MPE IC_{50} of 45μg/mL.

The urage of tamoxifen as positive control is a estrogen receptor α partial agonis, was regarded as first line drug in ER-α over expressed breast cancer treatment (Ao *et al.*, 2011; Fowler *et al.*, 2004). Tamoxifen, was also tested against MCF-7 cells by the same procedure. Tamoxifen had parabolic cytotoxic profile and had IC_{50} 47μM or equal to 12,65.10-7μg/mL by probit analysis. Tamoxifen had strong cytotoxic activity making MCF-7 cells broken into debris but MPE (Figure 2). This

could be explained that tamoxifen is pure chemical compound having spesific target on the cells. MPE also drived morphological changes in MCF-7 cells even it did not make the cells broken into debris. MPE contained various natural compounds so it did not have spesific target compared to THAT OFtamoxifen.

Assay then must be designed in other study to confirm molecular mechanism of MCF-7 cells. This study used double staining method using acridine orange and ethidium bromide. Viable cells were green and apoptotic cells were red. MPE showed apoptosis induction towards MCF-7 cells were dose-dependent.

This research used apoptotic cells 17 and 34μg/mL MPE to investigate apoptotic staining. MCF-7 cells were orange and those
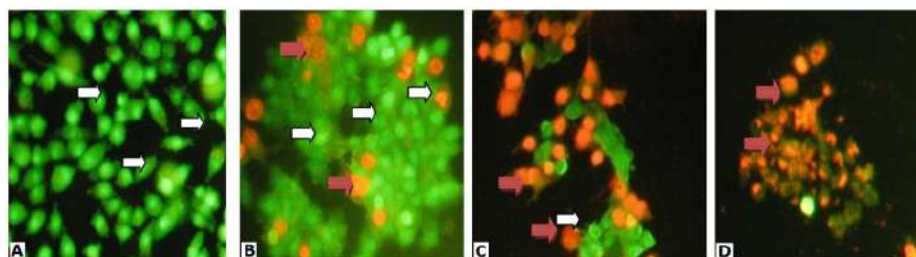
Figure 3. Apoptotic cells observation using double staining method under flourencence microscope using 400x magnification. (A)Untreated cells, (B)Cells treated 17μg/mL MPE (Cpoint C: MPE concentration should be "cells treated 34ug/mL MPE" (as described on the text) not 'cells treated 13ug/mL'; (D) Cells treated 12,65.10⁻⁷μg/mL tamoxifen ⇨ living cells; ⇨ apoptotic cells.
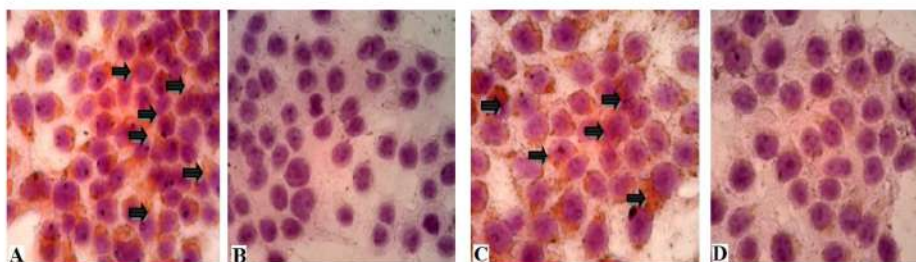

Figure 4. The observation of ER-α expression using immunocytochemistry method under light microscope using 400x magnification. (A)Untreated cells, (B) Untreated cells without antibody (C) MPE 17μg/mL treated cells (D) tamoxifen ➡ expressed ER-α

membrane integrity was disturbed (Figure 3). MPE has lower cytotoxic and apoptosis induction activity compare to that of tamoxifen, it might be due to complexity of MPE natural compound. These novel findings underline the benefit of in vitro study to elucidate molecular mechanisms of chemopreventive agent.

The molecular mechanism of apoptosis induction of MPE on ER-α was confirmed using immunocytochemistry method. Tamoxifen, a Selective Estrogen Receptor Modulators (SERMs), suppress ER-α expression. It down regulated ER-α by changing conformation of ER-α thus AF-2 domain is hide so AF-2 dependent co-activator hardly bind to the receptor (Brufsky, 2011). Untreated cells showed high expression of ER-α both in nucleus and cytoplasm. ER-α is located in nucleus and cytoplasm near cell membrane in the absense and presence of estrogen (Cheskis et al., 2007). MCF-7 cells treated with 34μg/mL made cells being fragmented into debris there fore this study MPE with concentratum of 17μg/ml, was used is detect ER-α expression. Even MPE had strong cytotoxic and apoptosis induction activity, it hardly suppressed ER-α expression. Is inhibited ER-α signaling pathway and might have other protein targets on MCF-7. There were 83 genes involved in ER-α signaling pathway in positive ER-α cell lines (Chisamore et al, 2009). Therefore, other genes involved in ER-α signaling pathway must be investigated.

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