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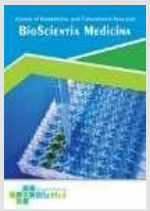

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Effect of Okra Fruit (*Abelmoschus esculentus*) Extract on Adenocarcinoma Mammae by Assessing Caspase-3 Expression and Apoptosis Index: An In Vivo Study

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A B S T R A C T

Background: Breast cancer is cancer in women with the highest incidence. Adriamycin-cyclophosphamide (AC) chemotherapy is a breast cancer therapy with good efficacy, but its effectiveness has not been maximized. The okra fruit extract is known to have anti-cancer effects from lectins, so it can be used as a complementary chemotherapy therapy to increase effectiveness with minimal side effects. **Methods:** This study is an experimental study using female rats Sprague Dawley strain, aged 4 weeks, were induced by DMBA to form mammary adenocarcinoma cells. Divided into groups; control (K): no treatment, treatment 1 (P1): AC chemotherapy (adriamycin 1.5 mg/time and cyclophosphamide 15 mg/time), treatment 2 (P2): okra extract 150 mg/kgBW/day, and treatment 3 (P3): combination of AC and okra extract. **Results:** The highest levels of caspase-3 and apoptosis index were found in the P3 group at 39.66±1.78 and 20.93±1.67, respectively. Giving green okra extract to chemotherapy agents can increase the anti-cancer effect by increasing the apoptosis index and caspase-3 levels significantly ($p<0.05$) compared to other groups. **Conclusion:** Okra fruit extract was able to increase the apoptosis response to adriamycin-cyclophosphamide chemotherapy in vivo, as indicated by the high expression of caspase-3 and apoptosis index.

1. Introduction

Breast cancer is the cancer with the highest incidence in the world, affecting women. Risk factors for breast cancer include non-modifiable factors such as race, ethnicity, genetics, and age and modifiable factors including diet, physical activity, hormones, and female reproductive factors. Circulating levels of endogenous sex steroid hormones such as estradiol (E2) are associated with breast cancer risk in postmenopausal women. In addition to promotive and preventive measures, cancer control efforts are also carried out through therapy. The main therapeutic measures consist of operative management, radiation, and chemotherapy. Chemotherapy is the treatment of choice in advanced breast cancer. Several combination regimens are most often used, including fluorouracil, adriamycin, and cyclophosphamide (FAC); fluorouracil, epirubicin, and cyclophosphamide (FEC); adriamycin and cyclophosphamide (AC);

cyclophosphamide, methotrexate, and fluorouracil (CMF). Neoadjuvant chemotherapy before surgery improves outcomes in patients with advanced breast cancer.[1-5]

Various methods are used to increase the efficacy of cancer chemotherapy, one of which is to increase the effect of apoptosis.[6] Various natural ingredients have been explored that have the effect of increased apoptosis in breast cancer.[7] The okra plant (*Abelmoschus esculentus* L.) is an annual shrub that is cultivated mostly in tropical and subtropical regions around the world. Apart from being used as food ingredients, okra seed extract and okra fruit have the ability to scavenge free radicals and have anti-cancer properties.[8] It is known that aqueous and methanol extracts from okra seeds and fruit have a wide toxicity window, so they are safe to use. The high content of flavonoids, isoquercetin, quercetin-3-O-gentiobiose, lectins, and pectins in okra extract is often studied for its anti-cancer benefits.[9-13] This study aimed to assess the effect of okra fruit extract on the apoptosis response to adriamycin-cyclophosphamide chemotherapy in vivo.

2. Methods

This study is an experimental study with a post-test-only approach with a control group design. The research subjects were female rats (*Rattus norvegicus*), Sprague Dawley strain, aged 5 weeks, and weight 100-150 grams. A total of 24 rats were grouped into 4 groups (each 6 rats/group), K Control group, rats induced by DMBA as much as 20 mg/kgBW, after the lumps developed were not given therapy, P1 : Treatment group 1, DMBA-induced rats were as much as 20 mg/kgBW, after a lump appeared, received AC chemotherapy (adriamycin-cyclophosphamide) 1.5 mg/times adriamycin and 15 mg/times cyclophosphamide, P2 : Treatment group 2, rats induced by DMBA as much as 20 mg/kgBW, after onset The lump received 150 mg/kgBW/day okra fruit extract, P3 : Treatment group 3, rats induced by DMBA as much as 20 mg/kgBW, after the lump appeared they received AC chemotherapy 1.5 mg/time adriamycin and 15 mg/time cyclophosphamide and green okra fruit extract as much as 150 mg/kgBW. This study was approved by the Medical and Health Research Ethics Committee, Faculty of Medicine, Universitas Diponegoro, Semarang, Indonesia.

4 kg of okra fruit that has been washed and air-dried at room temperature to meet the requirements of simplicia water content in general. The dried simplicia was then powdered until smooth and sieved with a B30 sieve. Making okra fruit ethanol extract is done by maceration, where okra fruit powder is put into a bottle, and ethanol is added until it is submerged, then stirred and left for 1 night. Take the filtrate and soak the residue again with ethanol until a clear filtrate is obtained. The obtained filtrate is separated by a rotary evaporator to obtain a condensed extract.

Experimental animals were killed with chloral hydrate, then placed on their backs on a fixation mat, and all four legs were fixed with a needle. The skin on the tumor was rubbed with 70% alcohol, and then an incision was made with straight scissors to remove the tumor. The tumor was placed in a small petri dish that had been washed with physiological saline and placed on ice. Then take/cut the tumor tissue that is still good, namely the part without necrosis (usually in the periphery if the tumor is large) as much as possible to produce a tumor slurry of at least 1 ml and put it in another small petri dish. The solid tumor residue was put into a formalin bottle for histopathological preparation. Adenocarcinoma slices were placed in buffered formalin solution (10% formalin solution in sodium acetate buffer until pH 7.0 was reached). Network fixation time 18-24 hours. After fixation is complete, the tissue is put in an aqua dest solution for 1 hour for the fixation solution removal process. Adenocarcinoma slices were placed in graded concentration alcohol. The tissue was then immersed in the alcohol-xylol solution for 1 hour and then in pure xylol solution for 2x2 hours. The tissue was put in liquid paraffin for 2x2 hours. The tissue is planted in solid paraffin, which has a melting point of 56-58^{0}C. Wait until the paraffin is solid. The tissue in paraffin was cut to a thickness of 6 microns with a microtome.

The tissue pieces were attached to a slide that had previously been smeared with polylysine as an adhesive. The tissue on the slide is heated in an incubator at 56-58^{0}C until the paraffin melts. Furthermore, immunohistochemical staining was performed to assess the expression of caspase-3.

After the data is collected, data cleaning, coding, and tabulation are carried out. Data analysis includes descriptive analysis and hypothesis testing in a descriptive analysis of tumor mass diameter and expression of Granzyme adenocarcinoma mammae presented in tabular form of mean and standard deviation. Then the data normality test was carried out with the Shapiro-Wilk test. The hypothesis test used is the One-Way ANOVA test, followed by the Post-Hoc Test to find out the differences between groups. The limit of the degree of significance is if $P < 0.05$ with a 95% confidence interval. Data analysis is done by SPSS software version 21.0 for Windows.

3. Results

Table 1 presents caspase-3 expression data between groups. Groups P1, P2, and P3 showed higher caspase-3 expression than group K. Groups P1 and P3 had higher caspase-3 expression than group P2. The combination of chemotherapy and okra fruit extract (P3) showed optimal potential in increasing caspase-3 expression. Table 2 presents the apoptosis index data between groups. Groups P1, P2, and P3 showed a larger apoptosis index than group K. Groups P1 and P3 had a larger apoptosis index than group P2. The combination of chemotherapy and okra fruit extract (P3) showed optimal potential in increasing the apoptosis index.

Table 1. Comparison of caspase-3 expression between groups.

Group	Caspase-3 expression (%) Mean ± SD
K	19.86 ± 3.75
P1	29.97 ± 2.63*
P2	22.25 ± 4.00*
P3	39, 66 ± 1.78*

*Post Hoc test LSD VS group K, $p < 0.05$.

Table 2. Comparison of apoptosis index expression between groups.

Group	Mean ± SD
K	2.58 ± 1.39
P1	18.05 ± 1.83*
P2	3.86 ± 1.31*
P3	20.93 ± 1.67*

*Post Hoc test LSD VS group K, $p < 0.05$.

4. Discussion

Caspase (cysteine-aspartate protease) is a molecule that actively contributes to apoptosis, inflammation, neuronal remodeling, and differentiation. In initiating apoptosis, there are two main pathways, namely, the extrinsic pathway and the intrinsic pathway. The extrinsic or death receptor (DR) pathway is activated in response to caspase-8 activation followed by caspase-3, whereas the intrinsic (or mitochondrial) pathway is triggered by mitochondrial cytochrome C release, leading to the formation of the Apaf-1 and cytochrome C complexes with the help of ATP, then activates caspase-9 followed by caspase-3.[14-16]

Okra contains lectins that have been extensively studied for their selective anti-cancer effects. Lectins have the effect of stopping the cell cycle in the G1 and G2/M phases, causing cell apoptosis. Another mechanism for the induction of apoptosis by lectins begins with their interaction with sugar-binding receptors on the plasma membrane, and endocytosis

occurs. Lectin vesicles go to the mitochondria to produce reactive oxygen species (ROS) and release cytochrome c from the mitochondria into the cytoplasm. ROS then activates several downstream effector molecules that regulate Bax and Bcl-2 and further trigger p21-Foxo1a-Bim-mediated apoptosis.[17] In another mechanism, plant lectins regulate NF-kB, ERK, and Ras to induce cell cycle arrest and apoptosis.[18,19] In another study, it was found that the administration of ethanol extract of okra fruit as much as 150 mg/kgBW, 300 mg/kgBW, and 450 mg/kgBW for 15 days could reduce the diameter and decrease the number of vascularization of breast cancer.[20]

5. Conclusion

Okra fruit extract was able to increase the apoptosis response to adriamycin-cyclophosphamide chemotherapy in vivo, as indicated by the high expression of caspase-3 and apoptosis index.

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