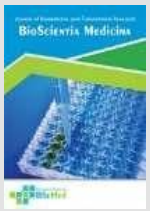
eISSN (Online): 2598-0580



Bioscientia Medicina: Journal of Biomedicine & Translational Research

Journal Homepage: www.bioscmed.com

Effect of Okra Fruit (*Abelmoschus esculentus*) Extract on Granzyme Expression and Tumor Mass Diameter of Adenocarcinoma Mammae: An In Vivo Study

Lugas Lion Halley[1*], Selamat Budijitno[2], Trilaksana Nugroho[3], Nani Maharani[4], Eriawan Agung Nugroho[5]

[1]Department of Surgery, Faculty of Medicine, Universitas Diponegoro, Semarang, Indonesia

[2]Division of Oncology Surgery, Department of Surgery, Faculty of Medicine, Universitas Diponegoro, Semarang, Indonesia

[3]Department of Ophthalmology, Faculty of Medicine, Universitas Diponegoro, Semarang, Indonesia

[4]Department of Pharmacology, Faculty of Medicine, Universitas Diponegoro, Semarang, Indonesia

[5]Division of Urology Surgery, Department of Surgery, Faculty of Medicine, Universitas Diponegoro, Semarang, Indonesia

ARTICLE INFO

Keywords:
Abelmoschus esculentus
Anti-tumor
Breast cancer
Chemotherapy
Granzyme

***Corresponding author:**
Lugas Lion Halley

E-mail address:
drlugaslionhalley@gmail.com

All authors have reviewed and approved the final version of the manuscript.

https://doi.org/10.37275/bsm.v6i13.660

A B S T R A C T

Background: Breast cancer is currently one of the most common types of cancer suffered by women, with the highest prevalence in all countries in the world. Herbal plants are thought to increase the immune system against tumors, one of which is okra (*Abelmoschus esculentus*) has the potential as an anti-tumor and anti-carcinogenic. This study aimed to assess the efficacy of ethanol okra extract on granzyme expression and tumor mass diameter in mammary adenocarcinoma of rats treated with adriamycin-cyclophosphamide chemotherapy. **Methods:** This study is an experimental study using 28 Sprague Dawley Rats. Data analysis was performed with SPSS version 21 to assess differences in granzyme expression and tumor size. **Results:** The combination of chemotherapy and okra fruit extract (P3) showed optimal potential in increasing granzyme expression and reducing mammary tumor size. **Conclusion:** The okra fruit extract has potential in the treatment of mammary carcinoma by increasing the expression of granzymes so as to reduce the size of breast carcinoma tumors in vivo.

1. Introduction

Breast cancer or mammary carcinoma is a malignancy in breast tissue that can originate from the ductal epithelium or its lobules. Breast cancer arises as a result of the proliferation of abnormal cells, which are the result of gene mutations with differentiation in shape, size, and function from the original body cells. This gene mutation is triggered by the presence of a foreign material that enters the body, such as radioactive, free radicals, or carcinogenic, originating from external and internal to the body. The threat of cancer is often increasing with unhealthy lifestyle changes such as smoking, consumption of fast food, environmental pollution, and depletion of the ozone layer. Breast cancer is currently one of the most common types of cancer suffered by women, with the highest prevalence in all countries in the world. The prevalence of breast cancer mortality in 2011 worldwide was 508,000 women.[1-5]

Management of breast cancer depends on the type and stage experienced by the patient. Surgery, radiotherapy, cytostatics, immunotherapy, and hormonal therapy are modalities of breast cancer therapy. Chemotherapy has side effects that damage the liver, kidneys, heart, and other organs of the body, as well as immunosuppressive effects. In addition, to the high cost of cancer treatment, people are starting to leave conventional cancer treatment modalities. People are trying to find other ways of treatment or complementary therapies for psychological and economic reasons, with minimal side effects in dealing with cancer. Complementary alternative medicine (CAM) is a therapy used as a complement or as an adjunct to conventional therapy. Complementary therapy can be combined or integrated with conventional therapy, so complementary therapy is also referred to as integrative therapy. One type of complementary therapy is the use of fruit or medicinal plants (herbs).[6-9]

Okra (*Abelmoschus esculentus*) is one of the most widely used medicinal plants. This plant began to be used to treat various diseases, such as cancer. Okra (*Abelmoschus esculentus* L. (Moench) is commonly known as lady finger, which is cultivated as an important vegetable crop because it acts as a source of carbohydrates, minerals, and vitamins such as potassium, sodium, magnesium, and calcium. In okra plants (*Abelmoschus esculentus),* there are lectins that have the potential as anti-tumor and anti-carcinogenic. Lectins are a heterogeneous group of proteins of non-immune origin capable of recognizing and reversibly binding mono or oligosaccharides and glycoconjugates. Lectins also play a role in modulating various cellular pathways, including cell apoptosis.[10-14] This study aims to explore the potential of okra fruit extract (*Abelmoschus esculentus*) against mammary carcinoma through granzyme activation and tumor size 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 5 weeks old, and weighing 100-150 grams. A total of 28 rats were grouped into 4 groups (each 7 rats/group), K : Control group, DMBA-induced rats at a dose of 20 mg/KgBW, 2 times a week, for 5 weeks via gastric probe, P1 : Treatment group 1 , rats induced with DMBA at a dose of 20 mg/KgBW, 2 times a week, for 5 weeks through a gastric probe, after a lump appeared, received AC chemotherapy (adriamycin-cyclophosphamide), P2 : Treatment group 2, rats induced with DMBA at a dose of 20 mg /KgBW, 2 times a week, for 5 weeks through a gastric probe, after a lump appears, receive *Abelmoschus esculentus* 150 mg/kg/day, P3 : Treatment group 3, rats induced by DMBA at a dose of 20 mg/KgBW, 2 times a week, for 5 weeks through a gastric probe, after a lump appeared, received AC chemotherapy and *Abelmoschus esculentus* 150 mg/kg/day. 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. The manufacture of ethanolic extract of okra fruit was done by maceration, where the okra fruit powder was put into a brown bottle and added ethanol until it was 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 granzyme.

After the data is collected, data cleaning, coding, and tabulation are carried out. Data analysis includes descriptive analysis and hypothesis testing. In the descriptive analysis, the diameter of the tumor mass and the expression of the Granzyme adenocarcinoma mammary were 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 was the One-Way ANOVA test, followed by the Post-Hoc Test to determine the differences between groups. The limit of the degree of significance is if $P < 0.05$ with a 95% confidence interval. Data analysis was carried out with SPSS software version. 21.0 for Windows.

3. Results

Table 1 presents the granzyme expression data between groups. Groups P1, P2, and P3 showed greater granzyme expression than group K. Groups P1 and P3 had higher granzyme expression than group P2. The combination of chemotherapy and okra fruit extract (P3) showed optimal potential in increasing granzyme expression. Table 2 presents data on tumor diameters between groups. Groups P1, P2, and P3 showed a smaller tumor size than group K. Groups P1 and P3 had a smaller tumor size than group P2. The combination of chemotherapy and okra fruit extract (P3) showed optimal potential in reducing tumor size.

Table 1. Granzyme expression between groups.

Group	Granzyme expression (%) (Mean ± SD)
K	15.38 ± 1.35
P1	25.00 ± 1.09*
P2	16.60 ± 1.16*
P3	26.52 ± 0 ,73*

* post hoc LSD VS K, $p < 0.05$).

Table 2. Tumor mass comparison between groups.

Group	Tumor mass diameter (%) Mean ± SD
K	6.45 ± 0.58
P1	5.05 ± 0.72*
P2	6.22 ± 0.56*
P3	3.63 ± 0.82*

* post hoc LSD VS K, $p < 0.05$).

4. Discussion

Lectins play a role in modulating various cellular pathways, including apoptosis. The biological activities of lectins include the ability to stop the cell cycle in the G1 and G2/M phases and activate caspase cascades. In addition, lectins in okra are excellent agents for studying glycosylation patterns. Post-translational modifications such as glycosylation play an important role in determining protein function. And may play a role in disease development.[15] Changes in the glycosylation pattern of cell surface proteins are associated with tumor development. Due to their cell surface expression, oligosaccharide epitopes of glycoproteins and glycolipids are recognized by the membrane-anchored carbohydrate recognition domains of various molecules, including lectins.[16] Binding of these lectins to oligosaccharide epitopes in tumor cells can activate cell death via apoptosis. Lectins from various sources have shown anti-tumor and anti-carcinogenic activity in increasing the function of mononuclear cells around cancer cells as effectors against cancer cells by looking at the products they secrete (e.g., granzyme and perforin).[17] Malignant transformation is often associated with changes in the pattern of cell surface glycosylation. Cancer development involves impaired cell cycle regulation, which, in turn, may contribute to altered expression of cell surface carbohydrates. Because of its anti-tumor effect, carbohydrate-binding lectins are the focus of attention in cancer biotherapy research. Breast cancer is the most frequently diagnosed invasive malignancy and the second leading cause of cancer-related death in women worldwide. Breast carcinogenesis may involve quantitative as well as qualitative changes in the expression of carbohydrates on the cell surface.[18] Therefore, lectins can provide a useful strategy for early detection and/or as a complementary therapy or adjuvant therapy for human breast cancer. Granzyme will activate endogenous procaspases in target cells. Caspase activity is part of the general apoptotic death pathway.[19] Caspases will activate DNAase, which causes DNA damage during apoptosis, so what will happen is the shrinking of the cell's organelles and nucleus.[20]

5. Conclusion

The okra fruit extract has potential in the treatment of mammary carcinoma by increasing granzyme expression so as to reduce the size of breast carcinoma tumors in vivo.

6. References

1. Bray F, Ferlay J, Soerjomataram I, Siegel RL, Torre LA, et al. Global cancer statistics 2018: GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 countries. CA Cancer J Clin. 2018; 68(6): 394–424.
2. Harbeck N, Penault-Llorca F, Cortes J, Gnant MN, Houssami P, et al. Breast cancer. Nat Rev Dis Primers. 2019; 5(1): 66.
3. Grivennikov SI, Greten FR, Karin M. Immunity, inflammation, and cancer. Cell. 2010; 140(6): 883–99.
4. Mantovani A, Allavena P, Sica A, Balkwill F. Cancer-related inflammation. Nature. 2008; 454(7203): 436–44.
5. Kohrt HE, Nouri N, Nowels K, Johnson D, Holmes S, Lee PP. Profile of immune cells in axillary lymph nodes predicts disease-free survival in breast cancer. PLoS Med. 2005; 2(9): e284.
6. Lin WW, Karin M. A cytokine-mediated link between innate immunity, inflammation, and cancer. J Clin Invest. 2007; 117(5): 1175–83.
7. Sica A, Allavena P, Mantovani A. Cancer-related inflammation: The macrophage connection. Cancer Lett. 2008; 267(2): 204–15.
8. Šmerák P, Šestáková H, Polívková Z, Štětina R, Langova M, et al. Antimutagenic effect of epigallocatechin gallate and its effect on the immune response in mice. Czech J Food Sci. 2006; 24(4): 180–92.

9. Simon SY, Gashua IB, Musa I. Genetic variability and trait correlation studies in okra *Abelmoschus esculentus* (L.) Moench. Agric Bio J North Am. 2013; 4(5): 532–8.
10. Esan AM, Masisi K, Dada FA, Olaiya CO. Comparative effects of indole acetic acid and salicylic acid on oxidative stress markers and antioxidant potential of okra (*Abelmoschus esculentus*) fruit under salinity stress. Sci Hortic. 2017; 216(7): 278–83.
11. Monte LG, Santi-Gadelha T, Reis LB, Braganhol E, Prietsch RF, et al. Lectin of *Abelmoschus esculentus* (okra) promotes selective anti-tumor effects in human breast cancer cells. Biotechnol Lett. 2014; 36(3): 461–9.
12. Wahyuningsih SPA, Pramudya M, Putri IP, Winarni D, Savira NII, Crude polysaccharides from okra pods (*Abelmoschus esculentus*) grown in Indonesia enhance the immune response due to bacterial infection. Adv Pharmacol Sci. 2018; 2018: 8505383.
13. Chen H, Jiao H, Cheng Y. In vitro and in vivo immunomodulatory activity of okra (*Abelmoschus esculentus*) polysaccharides. J Med Food. 2016; 19(3): 1–13.
14. Kiernan JA. Histological and histochemical methods: theory and practice. 5th ed. UK, Banbury: Scion Publishing, OX; 2015; 110–221.
15. Rudmann D, Cardiff R, Chouinard L, Goodman D, Kuutler K, et al. Proliferative and nonproliferative lesions of the rat and mouse mammary, Zymbal's preputial and clitoral glands. Toxicol Pathol. 2012; 40(6): 7S–39S.
16. Ouanouki A, Lamy S, Annabi B. Anthocyanidins inhibits epithelial-mesenchymal transition through a TGFbeta/Smad2 signaling pathway in glioblastoma cells. Mol Carcinog. 2017; 56(3): 1088–99.
17. Detrick B, Nagineni CN, Hook J. Cytokines: Regulators of immune responses and key therapeutic targets. In: O'Gorman MRG, Donnenberg AD, editors. Handbook of Human Immunology. New York: CRC Press, Taylor and Francis Group; 2008.
18. Mansingh DP, Pradhan S, Biswas D, Barathidasan R, Vasanthi HR. Palliative role of aqueous ginger extract on n-nitroso-n-methyl urea induced gastric cancer. Nutr Cancer. 2020; 72(1): 157–69.
19. Zhang HW, Hu JJ, Fu RQ, Liu X, Zhang YH, et al. Flavonoids inhibit cell proliferation and induce apoptosis and autophagy through downregulation of PI3K? mediated PI3K/AKT/mTOR/p70S6K/ULK signaling pathway in human breast cancer cells. Sci Rep. 2018; 8(1): 11255.
20. Verma S, Bahorun T, Singh RK, Aruoma O, Kumar A. Effect of *Aegle marmelos* leaf extract on N-methyl N-nitrosourea-induced hepatocarcinogenesis in Balb/c mice. Pharm Biol. 2013;1(10):1272–81.