The Effect of Ethanol Extract of Okra (*Abelmoschus esculentus* L.) Moench) on Tumor Growth in Breast Cancer Rats Model Induced by Benzo-a-Pyrene

Syarifah Riska Mela Putri[1], Salomo Hutahaean[1] and Syafruddin Ilyas[1], Widya Syahfitri[1] and Fitri Elizabeth[1]

[1]*Department of Biology, Faculty of Mathematics and Natural Sciences, Universitas Sumatera Utara, Medan, Indonesia*

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Abstract: Okra plant (*Abelmoschus esculentus* L. Moench)) is known as a medicinal plant that is traditionally used in treating various diseases. This study aims to investigate the effect of ethanol extract of okra seed pods on the growth of tumors in the rat breast cancer model. The experiment was carried out using a completely randomized design, with 5 treatments and 6 replications. These treatments were: 2 control groups (K- = normal group; K+ = breast cancer rat model due to benzo-a-pyrene or BAP injection), and 3 extract groups (breast cancer model rats were given okra extract at dose of 150, 300, or 450 mg/kg BW (body weight), respectively). The results showed that tumor growth occurred slowly in the first 3 months, but after that, the growth accelerated marked by an increase in tumor weight and tumor diameter. The average tumor weight due to BAP induction was 0.6 g, administration of the extracts of doses of 150 and 300 mg/kg BW gave no significant effect ($P>0.05$), but unexpectedly in doses of 450 mg/kg BW the average weight was 4.5 g ($P<0.05$). There is an indication that high-dose extracts stimulate tumor growth. The number of blood vessels has the same pattern as tumor growth. In the group of rats which were induced by BAP the number of blood vessels was 6 per visual area, whereas in the extract group the number increased by 9 to 10 vessels. The result suggest that A. esculentus extract may have an angiogenic effect at high doses.

1 INTRODUCTION

Breast cancer is one type of cancer causes a high mortality rate, especially against women. Breast cancer ranks second after cervical cancer in Indonesia. In the United States, breast cancer is the second leading cause of death in women (after lung cancer) (Price & Lorraine, 2006). Breast cancer is an important public health problem, because of its high mortality and morbidity. Based on research results in the Jakarta Breast Cancer in 2001 to 2003, of the 2,834 people who had breast lumps examined, 2,229 of them (78%) were benign tumors, 368 people (13%) were diagnosed with breast cancer and the rest were infections and breast congenital abnormalities (Djoerban Z, 2003).

The use of plants for medicinal purposes is common practice. Okra has been widely used in various traditional treatments. Even today, many communities use plants as the main source of treatment. Okra (*Abelmoschus esculentus*) is one of the most widely used plants for treatment. This plant began to be used to treat various diseases such as cancer, microbial infections, hypoglycemia, constipation, urinary retention and inflammation (Kumar, Patil, Patil, Patil, & Paschapur, 2009; Tomoda, Shimizu, Gonda, Kanari, & Yamada, 1989).

Okra, *Abelmoschus esculentus* L. (Moench) commonly known as "lady's finger" is cultivated as an important vegetable crop in tropical, subtropical and warm temperatures throughout the world. (Benchasri & Benchasri, 2012; S. Kumar et al., 2010; Lamont, n.d.; Ndunguru & Rajabu, 2004; Oyelade et al., 2003). Okra is rich in phenolic compounds and has high antioxidant activity. Okra has the potential to prevent several deadly diseases such as cardiovascular disease, type 2 diabetes, digestive diseases and some types of cancer (Gemede et al., 2015). Based on the latest research on lectins isolated from okra (*Abelmoschus esculentus*) tested in human breast cancer and

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fibroblast cells in the skin, okra has potential as an anti-tumor (Monte et al., 2014) and dried okra seeds have the potential to reduce tumor necrosis rates (Okada et al., 2010). This shows that okra has potential as an antioxidant contributor and promising chemopreventive agent for the treatment of diseases in human

Based on the description above, the research that will be carried out aims to find the effect of okra as an anti-tumor by looking at its effect on the growth of cancer cells by using the right dosage so that it can be a source of information for those who need to be developed into an alternative cancer treatment, especially cancer breast with raw materials derived from plants.

2 MATERIALS AND METHOD

This research has been carried out at the Laboratory of Structure and Development of Animals, Faculty of Mathematics and Natural Sciences, Laboratory of Organic Chemistry and Natural Materials, Faculty of Mathematics and Natural Sciences, Pathology Laboratory of Anatomy, Faculty of Medicine, University of North Sumatra.

2.1 Experimental Design

This study uses a completely randomized design (CRD) method which consists of 3 treatments with different concentrations and 2 treatments as controls. Both control and treatment each consisted of 6 replications so that there were 30 rats used.

2.2 Making of Okra Fruit Extract

The okra fruit was obtained from the Growth Center Laboratory of KOPWIL 1, North Sumatra. After being collected from the field, the okra fruit that has been washed clean is dried in an oven at 40oC until it meets the requirements of general moisture content. Simplisia that is dried and then made into powder until smooth and sieved with a B30 sieve. Making ethanol extract of okra fruit is done by maceration, ie okra fruit powder is put into a brown bottle and ethanol is added until submerged and then stirred and left for 1 night. Take the filtrate and re-soak the residue with ethanol until a clear filtrate is obtained. The filtrate obtained was separated with a rotary evaporator so that a thick extract was obtained.

2.3 Acclimatization of Experimental Animals

The experimental animals used were rats (Rattus sp.) Strains of healthy and fertile female Wistar aged 8-11 weeks with a weight of 200-250 g of 30 animals obtained from the North Sumatra Animal Disease Investigation Center Medan. Rats are kept in cages that are kept clean and feed and drink are done every day on an ad libitum basis. Handling of experimental animals by the requirements of the applicable code of ethics and before the research is conducted, an application for an Ethical Clearance to the Health Research Commission of the North Sumatra Region of Medan is submitted.

2.4 Extract Administration

Carcinogenic induction is carried out by injecting a solution of benzo (α) pyrene to the subcutaneous tissue of the Wistar strain rat in the mammary gland. Benzo (α) pyrene 50 mg/kg BW was dissolved in olive oil and given a single dose subcutaneously then observed the emergence of tumor mass in the breast of the rat by palpation (± 4 months), then continued with the test substance for 15 days.

The administration of treatment in this research is

a. negative control: without treatment
b. positive control: administration of Benzo [a] Pyrene (BAP) at a dose of 50 mg/kg body weight which will induce the growth of cancer cells in experimental animals
c. Treatment I: administration of Benzo [a] Pyrene (BAP) 50 mg/kg BW + ethanol extract of okra 150 mg/kg BW
d. Treatment II: administration of Benzo [a] Pyrene (BAP) 50 mg/kg BW + ethanol extract of okra fruit 300 mg/kg BW
e. Treatment III: administration of Benzo [a] Pyrene (BAP) 50 mg/kg BW + ethanol extract of okra fruit 450 mg/kg BW

2.5 Making the Histological Preparations

The histological preparation of the paraffin method begins with fixation, washing, dehydration, clearing, infiltration, embedding, slicing, attachment, deparaffination, staining, closing and labeling (Suntoro H, 1983).

2.6 Hematoxylin Eosin Staining

Hematoxylin-eosin staining is a standard coloring to determine the general structure of cells and tissues in an organ. The hematoxylin-eosin staining process starts from the deparaffination process followed by the rehydration process using multilevel alcohol then the preparations werw stained with haematoxylin and rinsed with distilled water for a few moments. The preparations are stained again with eosin and then proceed with the dehydration process. The preparations were clarified with xylol solution and continued with the mounting process (Suntoro, 1983).

2.7 Test Parameter Analysis

2.7.1 Visual Morphological Analysis

The morphological observations in this study were body weight, tumor weight, tumor diameter.

2.7.2 Histological Analysis of Mammary Gland

The histological part of the mammary gland observed in Hematoxylin-eosin staining is vascularization of the mammary gland. Observations were made by counting the number of blood vessels formed in the rat breast organs using a microscope with a magnification of 100x. Observations were made as many as 5 fields of view.

3 RESULTS AND DISCUSSION

3.1 Body Weight

Based on research that has been done, the administration of ethanol extract of okra fruit (*Abelmoschus esculentus* (L.) Moench) to the bodyweight of rats, the highest weight was the negative control group in which the weight was 261.6 g at week 13 and the lowest body weight was P2 group which was 201.3 g at week 6. The results of observations of body weight of rats (Rattus sp.) can be seen in Figure 1.

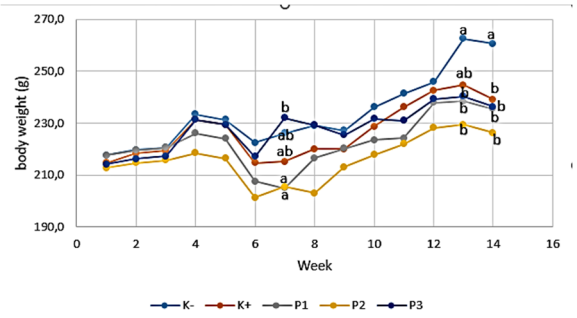

Figure 1: Effect of Okra Fruit Ethanol Extract on Body Weight of Rats (Rattus sp.) K- = Negative Control (mice not trained); K + = Positive Control (BAP distribution); P1, P2 and P3 = Treatment with ethanol extract concentrations of 150 mg/kg okra fruit, 300 mg/kg, and 450 mg/kg; unit in grams (g).

Statistical analysis showed that at week 6 the body weight of the P3 treatment group was significantly different (P <0.05) to the body weight of the treatment group P1 and P2 rats but not significantly different to the positive and negative control groups. At the 13th week during the administration of okra fruit ethanol extract, an increase in body weight of rats in which the negative control group was significantly different (P <0.05) with the treatment groups P1, P2, P3 and not significantly different from the positive control group. At the 14th week after administration of okra fruit ethanol extract there was a decrease in body weight in which the negative control group was significantly different (P <0.05) with positive control and treatment groups P1, P2 and P3.

Week 4 to week 10 there is an increase and decrease in body weight that is volatile. Inflammation in mice after BAP administration does not affect the body weight of mice but the physiological conditions of rats and other external factors such as weather can affect the body weight of mice. The size of mammary gland tumors in mice affects the body weight of mice. It can be seen in Figure 1 that there was a gain in weight at week 13 due to a significant increase in tumor size from the previous weeks so that the tumor mass affected the body weight of mice. In recent years, there have been reports showing that mice became fat in carcinogenicity studies and tumor growth in these mice. Selection, disease control, improved diet, and better control of environmental conditions led to an increase in body weight and life span of mice used in long-term toxicity studies over the past 2 decades (Rao et al., 1990). There was a decrease in body weight of rats in the 14th week after giving ethanol extract of okra fruit due to the size of the tumor that

began to shrink so that the tumor mass also decreased which would affect the body weight of the rat. Anorexia and weight loss are part of end-stage cancer syndrome which is a major cause of morbidity and mortality in cancer (Johnen et al., 2007).

3.2 Tumor Weight of Mammary Gland

Based on research that has been done, the administration of ethanol extract of okra fruit (*Abelmoschus esculentus* (L.) Moench) on mammary gland tumor weight (Rattus sp.), it can be seen that the highest tumor weight was the P3 group in which the weight was 4.51 g and the lowest was the P1 group which was 0.5 g. The results of observations of mammary gland tumor weight (Rattus sp.) Can be seen in Figure 2.

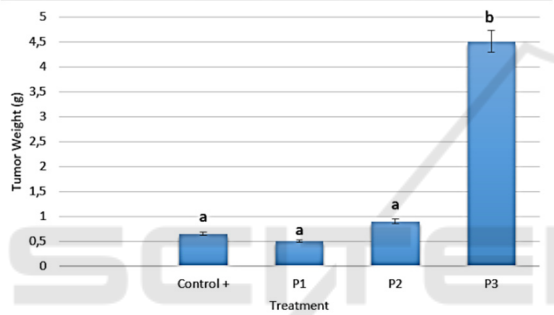

Figure 2: Effect of Okra Fruit Ethanol Extract on Tumor Weight of Mammary Gland of Rats (Rattus sp.) K- = Negative Control (mice not trained); K + = Positive Control (BAP distribution); P1, P2 and P3 = Treatment with ethanol extract concentrations of 150 mg/kg okra fruit, 300 mg/kg, and 450 mg/kg; unit in grams (g).

The results of statistical analysis showed that the tumor weight of the P3 group was significantly different (P <0.05) to the weight of the positive tumor group, the treatment groups of P1 and P2. The increase in tumor weight is caused by the continued division of cells. Proto-oncogenes are genes that help cells grow normally. When proto-oncogenes mutate (change) or there are too many copies, cells grow out of control. This can cause cancer. When this gene changes, it no longer suppresses the growth of abnormal cells and cancer is more likely to develop (American Cancer Society, 2018).

3.3 Tumor Diameter of Mammary Gland

Based on research that has been done, the administration of ethanol extract of okra fruit (*Abelmoschus esculentus* (L.) Moench) on mammary gland tumor diameter (Rattus sp.), it can be seen that the highest tumor diameter was the P3 group at week 13 which was 1.63 cm and the lowest tumor diameter was the P2 group at 13 weeks which a length was 0.64 cm. The results of observations on the diameter of mammary gland tumors can be seen in Figure 3.

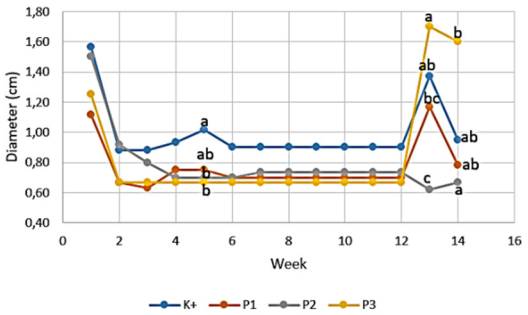

Figure 3: Effect of Okra Fruit Ethanol Extract on Tumor Diameter of Mammary Gland of Rats (Rattus sp.) K- = Negative Control (mice not trained); K+ = Positive Control (BAP distribution); P1, P2 and P3 = Treatment with ethanol extract concentrations of 150 mg/kg okra fruit, 300 mg/kg, and 450 mg/kg; unit in centi meter (cm).

Statistical analysis showed that at week 5th the diameter of the mammary gland tumor mice in the positive control group was significantly different (P <0.05) to the diameter of the mammary gland tumor in the treatment group P2 and P3 but it was not significantly different in the treatment group P1. There was a decrease in the diameter of the mammary gland in the second week after BAP administration because at this stage the swollen mammary glands due to inflammation that formed after injection had begun to shrink. Inflammation can cause tumor growth and development through its influence on cell proliferation, tumor survival and metastasis (Buckland et al., 2014).

In the following weeks, tumors began to form marked by the presence of thickening in the skin of the mammary gland of mice. The primary sign of a tumor in the mammary gland is the irregular border of the tumor due to the infiltration process into the surrounding tissue or unclear boundary (comet sign) and also changes in tumor mass both in size, consistency, and shape. The only way to diagnose gold (gold standard) in breast cancer is by histopathological examination, with this type of histology known (type), sub-type and cellular grading and core grading (Ramli, 2015). One of the histological examination of tumors is the determination of Ag-Nor grains in which the

Nucleolar Organizing Region (NOR) is a place of ribosomal biogenesis in the cell nucleus whose numbers increase with the increase in the activity of cell protein biosynthesis (Hutahaean et al., 2009). At week 12 there was a very significant change in tumor size and metastasis that was marked by the growth of tumors in other organs both in other mammary glands and in the abdomen of mice. At the 13th week during the administration of okra fruit ethanol extract, a decrease in the diameter of the tumor in mammary glands occurred. Lectins contained in okra fruit can inhibit the growth of mammary gland tumor cells in vitro (Monte et al., 2014).

3.4 Tumor Vascularisation of Mammary Gland

Based on research that has been done, the administration of ethanol extract of okra fruit (*Abelmoschus esculentus* (L.) Moench) against vascularisation in mammary gland tumor can be seen that the highest number of blood vessels is found in the P3 group which amounted to 10.1 and the lowest blood vessel is the negative control group which amounted to 6.5. The results can be seen in Figure 4.

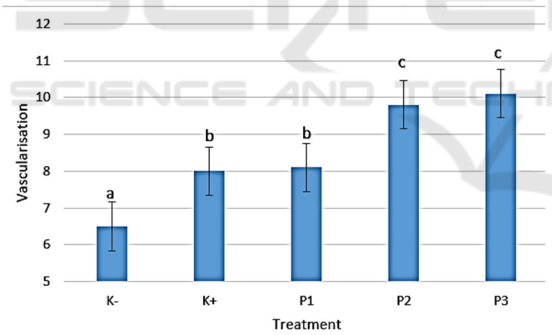

Figure 4**:** Effect of Okra Fruit Ethanol Extract on Tumor Vascularisation of Mammary Gland of Rats (Rattus sp.) K- = Negative Control (mice not trained); K + = Positive Control (BAP distribution); P1, P2 and P3 = Treatment with ethanol extract concentrations of 150 mg/kg okra fruit, 300 mg/kg, and 450 mg/kg.

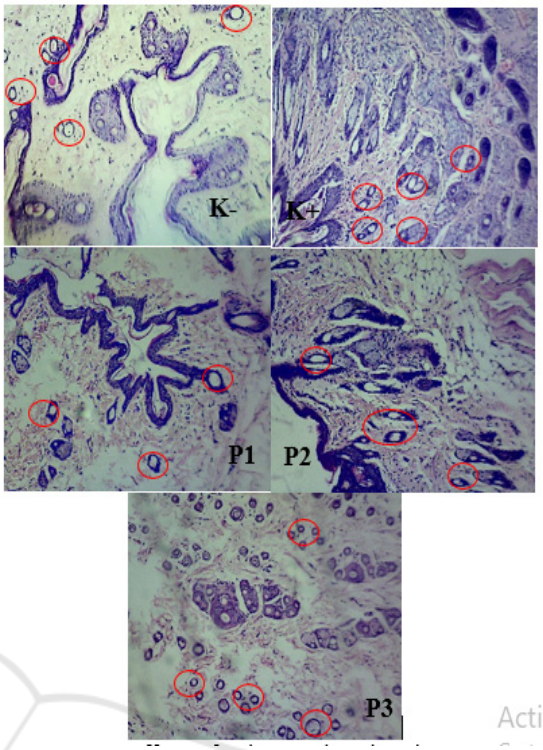

Figure 5: Effect of Okra Fruit Ethanol Extract on Tumor Vascularisation of Mammary Gland of Rats (Rattus sp.) K- = Negative Control (mice not trained); K + = Positive Control (BAP distribution); P1, P2 and P3 = Treatment with ethanol extract concentrations of 150 mg/kg okra fruit, 300 mg/kg, and 450 mg/kg. Vascularisation is indicated by a red circle.

The analysis showed that the number of blood vessels formed in the negative control group was significantly different ($P < 0.05$) for all treatment groups. This is in line with the tumor mass of mammary glands, where the greater the tumor mass, the more blood vessels are formed. One of the secondary features of the presence of a tumor in the mammary gland is characterized by an increase in blood vessels (Ramli, 2015). Vascularization or also called angiogenesis is the formation of new blood vessels originating from existing blood vessels. Under pathological conditions, angiogenesis is needed in the growth process of solid tumors and the process of metastasis (Hicklin & Ellis, 2005; Lee S. Rosen, 2002). Tumors require angiogenesis to grow above 1-2 mm^3 in size (Lee S. Rosen, 2002). Angiogenesis is needed for oxygen supply, nutrients, growth factors and hormones, proteolytic enzymes, influencing hemostatic factors that control coagulation and fibrinolytic systems and the spread of tumor cells to distant sites (Hicklin & Ellis, 2005).

4 CONCLUSIONS

The results showed that tumor growth occurred slowly in the first 3 months, but after that, the growth accelerated marked by an increase in tumor weight and tumor diameter. The average tumor weight due to BAP induction was 0.6 g, administration of the extracts of doses of 150 and 300 mg/kg BW gave no significant effect (P>0.05), but unexpectedly in doses of 450 mg/kg BW, the average weight was 4.5 g (P<0.05). There is an indication that high-dose extracts stimulate tumor growth. The number of blood vessels has the same pattern as tumor growth. In the group of rats which were induced by BAP the number of blood vessels was 6 per visual area, whereas in the extract group the number increased by 9 to 10 vessels. The result suggest that A. esculentus extract may have an angiogenic effect at high doses.

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