

RESEARCH ARTICLE

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# Chemopreventive Effect of Dietary *Maranta arundinacea* L. Against DMBA-Induced Mammary Cancer in Sprague Dawley Rats Through the Regulation of Autophagy Expression

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## Abstract

**Background:** Breast cancer prevention still needs to be improved. Calorie restriction is thought to prevent breast cancer through the induction of autophagy. *Maranta arundinacea* L. (MA) has the potential for calorie restriction because it contains high fiber. This research aimed to observe the effect of dietary MA against dimethylbenz(a)anthracene (DMBA)-induced mammary cancer in Sprague Dawley rats related to autophagy. **Methods:** Twenty-five Sprague Dawley rats were randomly divided into five groups: 1) control group without DMBA-induced with a standard diet, 2) 20 mg/kg BW of DMBA two times a week for five weeks with a standard diet, 3) DMBA and diet modification with 30% of MA, 4) DMBA and diet modification with 45% of MA, and 5) DMBA and diet modification with 60% of MA. Examination of the nodule was conducted once every week for 22 weeks. Breast tissue/tumor examination underwent histology examination with hematoxylin-eosin. Examinations of immunohistochemical staining against Beclin1, LC3B, and SQSTM1 were conducted to reveal autophagy. The difference of autophagy protein expression was analyzed using One way ANOVA with 95% confidence level and significance set as  $p < 0.05$ . **Results:** Cancer was detected in four rats of DMBA standard diet, two rats of 30% MA, one rat of 45% MA. No cancer was detected in the rats of control and rats with 60% of MA group. The Beclin1 expressions showed that the 60% of MA group had the highest score ( $2.5 \pm 0.52$ ) followed by the 45% of MA group ( $1.87 \pm 0.49$ ), control group ( $1.77 \pm 0.11$ ), 30% of MA group ( $1.28 \pm 0.75$ ), and DMBA with standard diet had the lowest score ( $1.28 \pm 0.91$ ). The difference of Beclin1 expressions was statistically significant ( $p$ -value=0.03). However, the difference of the LC3B expressions ( $p$ -value=0.11) and SQSTM1 expressions ( $p$ -value=0.225) were not statistically significant. **Conclusion:** Dietary modifications with MA potentially prevent breast cancer and induce initiation of autophagy.

**Keywords:** *Maranta arundinacea*- breast cancer- DMBA- Arrowroot- autophagy

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## Introduction

Cancer is one of the diseases that has become a major global health problem and is the second cause of death after cardiovascular diseases in the non-communicable group (Wang et al., 2016). Most cases of cancer found in women are breast cancer (Bray et al., 2018). The number of cancer cases has been reported to keep increasing in the world including in Indonesia (Depkes, 2016). Meanwhile, therapy for breast cancer is still facing significant problems, except for patients in the early stage. There are still some breast cancer patients with type Luminal A who are failing in the therapy with

anti-hormonal administration (Viedma-Rodríguez et al., 2014). A systematic review involving 6,980 patients using anti-HER2 found about 66 side effects with the commonest symptoms of diarrhea (29%), and skin rash (22%) (Sodergren et al., 2016). Several models have been developed for early detection of cancer, but it is not always available for every region (Li and Shao, 2015; Anna, 2016; Savery et al., 2017; Anwar et al., 2018). It is hard to identify people with the highest risk for treatment so that the incidence would decline (NCCN, 2015). Breast cancer prevention becomes equally important. Lifestyle-related risk factors are more likely to be intervened (Depkes, 2016). One of the risks related to lifestyle is a diet of

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overeating that causes obesity or an increase in body mass index (BMI). Preventing the high incidence of patients with obesity in Canada allowed a significant decrease in cancer cases (Dobbins et al., 2013). At least 24 researches on calorie restriction in animal models showed that 23 demonstrated retardation effects against breast cancer (Lv et al., 2014; Chen et al., 2016). Observation research on a low-calorie diet involving 40,318 subjects which included 38,660 women who after consuming a diet with more than 2,406 or 2,084 kcal/day and observed for 16.4 or 10 years showed increased risk for breast cancer compared to a diet with less than 1,630 or 1,316 kcal/day (HR:1.18; 95% CI =1.02–1.36; p = 0.02) and (RR:1.25; 95% CI:1.02-1.53; p = 0.03), respectively (Chang et al., 2006; Silvera et al., 2006; Gouel and Guimbar, 2017).

Calorie restriction can be attempted by swapping the source of carbohydrates. Arrowroot or *Maranta arundinacea* L. (MA) has carbohydrate content and both high soluble and insoluble fiber (Utami, 2008). Soluble fiber can increase food volume causing food to thicken and inhibit gastric emptying causing a person to feel full. This satiety makes a person to not want to eat and to limit their intake. Soluble fiber also inhibits digestion, carbohydrate, and fat absorption. MA contains resistant starch causing fat absorption inhibition; resistant starch can bind with bile acids which can inhibit fat absorption. Those two factors indirectly decrease calorie intake. Besides these benefits, ethanol extract of MA contains phenols, flavonoids, steroids, tannins, and glycosides (Nishaa et al., 2013), and it shows the high activity as free radicals against DPPH, ABTS, hydrogen peroxide, and nitric oxide (Nishaa et al., 2012). The antioxidants in MA can prevent damage caused by reactive oxygen species (ROS) and are used as an anti-inflammatory agent (Rajashekhara et al., 2014).

The prevention approach can be done by inhibiting the mechanism of breast cancer. This mechanism can happen through the P13/AKT line. Calorie restriction of breast cancer has been proved to be able to prevent calorie restriction through this line (Jiang et al., 2008; Rogozina et al., 2014). Calorie restriction inhibits MTOR1 and activates autophagy regulatory proteins such as Beclin 1, ATG, and LC3, so that autophagy occurs. Autophagy can maintain the quality of organelles and homeostasis of cells by preventing the accumulation of organelles containing toxic substances through recycling (Kopeina et al., 2016). Through ATM and p53 stimulation, autophagy is also speculated to be able to activate DNA repair, so it might be possible to inhibit genomic instability (Liu et al., 2015; Eliopoulos et al., 2016). In contrast, research showed that the gene damage related to autophagy such as Beclin1 stimulates tumorigenesis of breast cancer (Zarzynska, 2014).

The autophagy process occurs through the following stages: 1) initiation, 2) vesicle nucleation (formation of a phagophore), 3) vesicle elongation (formation of an autophagosome), 4) vesicle fusion (formation of an autophagolysosome), and 5) degradation (Levy et al., 2017). Beclin1 is the main initiator that will recruit key autophagy proteins (Marquez and Xu, 2012). LC3B localized to the autophagosome and autolysosome membranes is a marker for autophagy activity. SQSTM1

can be degraded during the autophagy process. The presence of this protein accumulation is usually considered as an indication of autophagy inhibition (Klionsky et al., 2016). The mechanism related to autophagy activation as the result of MA administration on breast cancer as far as the researchers' knowledge has not been investigated yet. This research aimed to see the effect of MA administration in a diet toward dimethylbenz(a)anthracene (DMBA)-induced Sprague Dawley rats' mammary tumorigenesis and its mechanism that is related to autophagy.

## Materials and Methods

### Materials

Standard feed production (AIN93) and MA modification were conducted in the Biochemistry Lab of Faculty of Medicine, Public Health and Nursing, Universitas Gadjah Mada (FKKMK UGM). MA flour and other materials such as protein, fat, fiber, mineral, and vitamin were mixed. The carbohydrate source of standard feed was exchanged with MA as much as 30%, 45%, and 60%. MA modification feed was made into pellets matching the standard feed.

### Subject description

This research was approved by the Medical and Health Research Ethics Committee of the FKKMK UGM with number KE/FK/1216/EC/2019. White rat type Sprague Dawley was obtained from LPPT UGM. Inclusion criteria were: rats that have never been used for research before, female, healthy, and no defects (have no visible anatomic abnormality), weighing around 37-73 grams, and aged four weeks. The exclusion criteria were that the rats had symptoms of illness or died before the mammary tumor appeared. The number of Sprague Dawley rats used in this research was based on the formula:  $E=10-20=$  total number of animals – total number of groups;  $20= 25$  rats – 5 groups (Charan and Biswas, 2013). The total number of rats used for the research was 25. Rats were randomized using a computer into five groups: control group (without DMBA-induced), DMBA group with AIN93 feed, and DMBA group with carbohydrate modification of 30%, 45%, and 60% of MA flour. The rats were kept according to animal welfare in a room with temperature  $23\pm 2$  °C, 12 hours light and dark adaptation, air humidity 70 – 80%, and an always-clean cage. At the beginning of the first week, all rats were given standard feed and drink ad libitum. In the second week, rats from groups 3, 4, and 5 were given MA feed. In the third week, rats from groups 2, 3, 4, and 5 were DMBA-induced. DMBA was administrated orally two times a week for five weeks with a dose of 20 mg/kg BW dissolved in corn oil. After DMBA induction, every week, rats were examined for the onset and the number of nodules. The size of nodules was measured with a caliper ruler since the first week after the last DMBA administration (rats aged ten weeks) for 22 weeks. In week 29, the experimental animals were euthanized for taking the nodules or mammary glands. The nodules were then weighed. The tumor volume was measured in cm<sup>3</sup> using the formula:  $\pi/6 \times \text{length of tumor} \times \text{width of tumor}^2$  (de Lorenzo et al., 2011).

*Tissue processing*

The fixation of nodules or mammary glands used 10% buffer formalin for 48 hours. The tissue was trimmed and put in a cassette. The tissue processing was conducted overnight for the dehydration process with serial alcohol, xylol, and paraffin. Paraffin wax embedding before section sliced for 4µm thickness. The histological slide was stained the hematoxylin-eosin. Before mounting, preparations were dehydrated again using serial alcohol and xylol.

*Immunohistochemical staining*

Deparaffinization was done by soaking the sample in xylene three times for 5 minutes and continued with rehydration using 100%, 95%, 80%, 70% of alcohol, and aquadest. In citric buffer, the slide was heated at the temperature of 95°C for 20 minutes. Slides were moved into a humidity chamber and then washed with TBS 1x1,000 µL three times for 5 minutes. Endogenous peroxidase inhibition was done with H<sub>2</sub>O<sub>2</sub> 3% for 20 minutes. Slides were washed with TBS 1x1000 µL once for 5 minutes. Blocking background was done with serum block (@Fine test Cat no.IHC0007) for 60 minutes at 37°C. The first antibodies are Beclin 1:300 (@ABclonal Cat NoA7353), LC3B 1:300 (@ABclonal Cat No.A7198), SQSTM1 1:200 (@ABclonal Cat No.A11483); the procedures were conducted for an hour at room temperature. Slides were washed with TBS 1x1,000 µL three times for 5 minutes and Poly-HRP-Goat Antirabbit (@ Fine test Cat no. IHC0007) for 45 minutes at 37°C. Slides were washed with TBS 3x1,000 µL four times for 5 minutes and then were dripped with DAB 1:20 solutions for four minutes. Washing using running water was conducted for 10 minutes in a glass jar. Sample slides were soaked in glass jars containing hematoxylin for two minutes, lastly washed with running water for 5 minutes continued with dehydration, clearing, and mounting. Autophagy protein expressions: Beclin 1, LC3B, SQSTM1 were counted for its number of cells with brown cytoplasm compared to all numbers of cells in 10 fields of views using Raster image software with the semi-quantitative histologic score (HSCORE) system. HSCORE formula =  $\sum Pi (i + 1)/100$ , in which i = painting intensity in which value 1 (weak), 2 (medium), 3 (strong), and Pi = cells percentage (0-100%) that is painted positive for each intensity (Germeyer et al., 2014).

*Data Analysis*

The tumor in behalf of incidence, tumor multiplicity (number of nodules/rats), an average of nodule weight, the volume of the nodule, and time of nodule appearance were shown with the frequency table. Histological features were assessed descriptively. Expression of autophagy protein was analyzed by two readers for ten slides randomly and being assessed with Intraclass Correlation Coefficient (ICC) test. The expression difference of autophagy protein was assessed with the One way ANOVA test continued with Post hoc using Tukey test with confidence level 95% and significance set as p<0.05. Before conducting the ANOVA test, the normal distribution was determined using the Shapiro Wilk test and homogeneity/variance using the Levene test.

**Results**

*Body Weight and Amount of Feed*

The bodyweight of Sprague Dawley rats at the beginning of treatment and after adaptation had no difference among groups. All groups' body weights increased along with the age but were relatively constant after 14 weeks of treatment (Table 1). Bodyweight had no difference from the controls during and some weeks after induction using 20 mg/kg BW of DMBA two times a week for five weeks. Generally, the MA diet did not change the development of weight gain in rats, except for group 30% MA. The growth of this group was slower, and in the 14<sup>th</sup> week, this group was seen to have the lightest body weight compared to other groups. At week 26, the bodyweight of the DMBA standard diet group and 30% MA started to decrease. Meanwhile, the bodyweight of the 45% MA group decreased at the last treatment. At week 29, DMBA standard diet, 30% and 45% MA groups had lighter bodyweight than controls or the 60% MA group.

In the first week of adaptation, the amount of feed did not differ significantly among groups. However, at week 3, 45% and 60% of MA groups ate in lesser amounts. In week 4, the feed amount of 30%, 45%, and 60% MA groups was less than the standard diet groups. At week five, the 60% MA group had started to eat more. The 45% MA group ate less until week 8, while the 30% MA group was until week 9 (Table 2). The MA groups ate less fed in the early weeks but their weight did not differ significantly from standard diet groups. Then, starting from week ten until week 26 the feed amount of all groups did not differ

Table 1. Body Weight among Groups before, during and after DMBA Induction and Diet Modification

Groups	n	Body weight Week 1 (mean±SD)	Body weight Week 2 (mean±SD)	Body weight Week 14 (mean±SD)	Body weight Week 26 (mean±SD)	Body weight Week 29 (mean±SD)
Control	5	56.41±10.72	71.73±15.84	178.2±23.11	204.4±21.67	196.00±24.83
DMBA standard diet	5	55.19±11.19	69.31±18.93	179.8±13.71	161.8±20.71*	150.75±19.17*
DMBA MA 30%	5	57.77±6.61	73.90±8.21	149.0±22.53	166.4±18.03*	158.22±18.45*
DMBA MA 45%	5	55.72±11.72	68.78±16.61	169.60±23.32	175.8±33.67	160.20±27.08*
DMBA MA 60%	5	58.34±18.1	69.52±15.08	177.4±22.13	181.0±30.15	181.00±16.35
p value		0.98	0.53	0.15	0.083	0.026

Notes: Body weight (gram); \*p<0.05 compared to control; n, number of animal study; SD, standard deviation

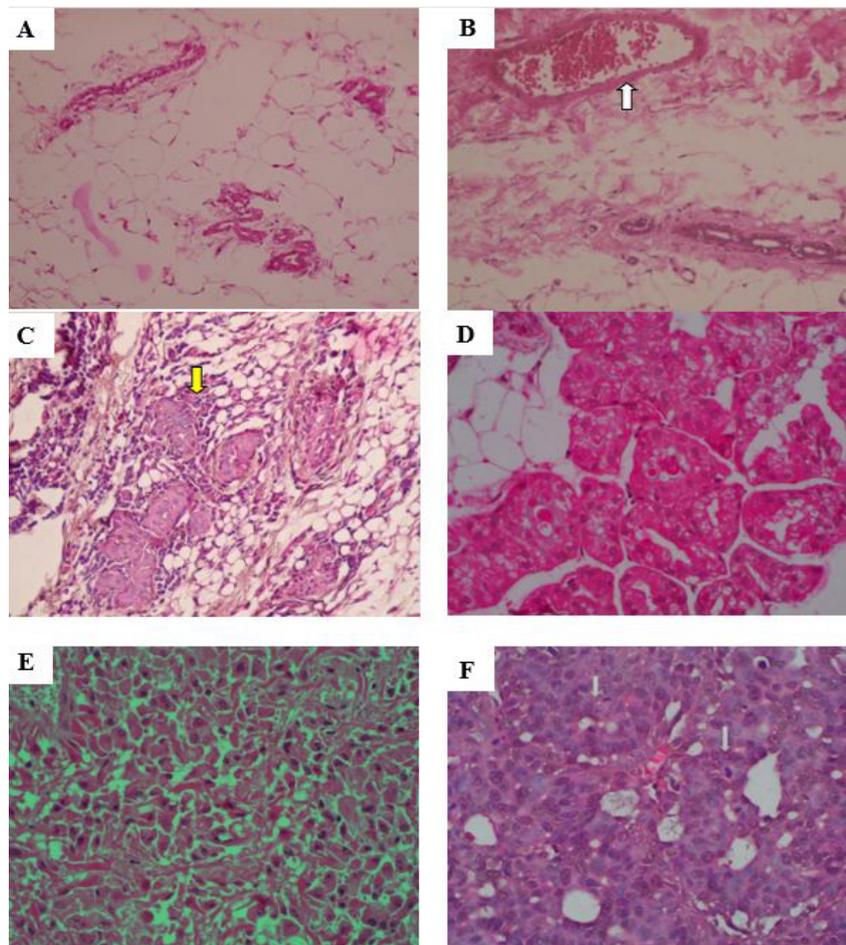


Figure 1. Histopathology of Nodule and Mammary Gland after Given DMBA 20 mg/kg BW Two Times a Week for Five Weeks on Standard Diet Group also 30%, 45% and 69% of MA. A. normal mammary gland: no proliferation (control); B. mammary gland with inflammation (yellow arrow showed the congestion) on one rat DMBA standard diet group; C. mammary gland with inflammation (arrow: polymorphonuclear cell) on DMBA 45% MA group; D. mammary gland experiences hyperplasia (60% MA group); E. carcinoma with pleomorphic appearance (DMBA standard diet group); F. carcinoma with increase in mitosis (white arrow) on 30% MA group; Magnification 400X

significantly. However, in week 27, the DMBA standard diet group ate less compared to the other groups.

*Nodule and Histopathological Status*

After 22 weeks of observation, induction of DMBA of 20 mg/kg BW twice a week for five weeks could cause breast nodules to develop in the groups of standard diet, 30% and 45% MA. Percentage of nodule growing (incidence) and multiplicity (number of nodules/rats) were

highest in the group of DMBA standard diet (100% and 1.17). All of the rats in this group formed a nodule. In the group of DMBA standard diet, one rat had two nodules, and four rats had one nodule. Administration of 30%, 45%, and 60% MA could cause a decrease in incidence and multiplicity of breast nodules, and all test animals of group 60% MA did not form breast nodules. The nodules volume and weight started from the biggest were DMBA standard diet group, DMBA group with 30%, 45% MA

Table 2. The Amount of Feed among Groups before, during and after DMBA Induction and Diet Modification with 30%, 45% and 60% of MA

Groups	n	Amount of feed	Amount of feed	Amount of feed	Amount of feed	Amount of feed
		Week 1 (mean±SD)	Week 2 (mean±SD)	Week 4 (mean±SD)	Week 16 (mean±SD)	Week 28 (mean±SD)
Control	5	48.261±13.15	57.84±7.78	60.04±2.35	54.54±13.01	59.12±6.27
DMBA standard diet	5	44.84±10.82	51.98±1.96	55.82±5.33	47.265±10.073	48.95±5.83*
DMBA MA 30%	5	47.28±7.81	49.14±2.78	47.84±8.37*	46.76±9.52	56.5±8.76
DMBA MA 45%	5	43.92±6.93	46.2±10.7*	43.96±5.91*	48.28±5.9	54.04±16.96
DMBA MA 60%	5	50.0±8.2	45.98±5.79*	45.92±13.47*	56.182±13.55	58.38±13.36
p value		0.85	0.06	0.021	0.52	0.49

Notes: Amount of feed in a week (gram); \*p<0.05 compared to control; n, number of animal study; SD, standard deviation.

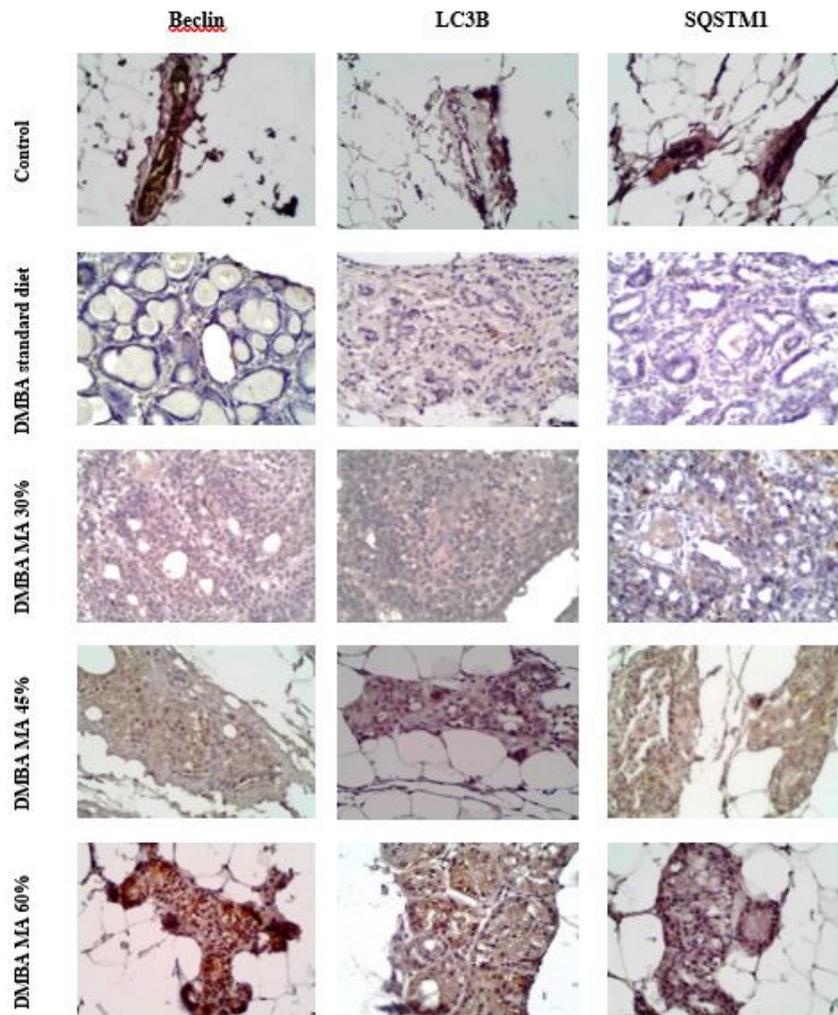


Figure 2. Expression of Autophagy Protein. Expressions of Beclin 1, LC3B and SQSTM1 of control group and DMBA 60% tend to be higher (brown cytoplasm). Expressions of Beclin 1, LC3B and SQSTM1 on DMBA standard diet group and 30% MA are rare (no brown cytoplasm), Magnification 400X

Table 3. Profile of Mammary Nodules after DMBA Induction

	Control	DMBA standard diet	DMBA MA 30%	DMBA MA 45%	DMBA MA 60%
N	5	5	5	5	5
Nodules					
Number	0	5	2	1	0
Tumor in behalf of incidence	0	100%	40%	20%	0
Total	0	6	2	1	0
Multiplicity	0	1.2	1	1	0
Weight of nodules (gr)					
Total	0	10.89	3.44	1.72	0
Mean	0	2.19	1.72	1.72	0
Volume of nodules (cm <sup>3</sup> )					
Total	0	18.11	6.09	2.66	0
Mean	0	3.62	3.04	2.66	0
Time of nodule (week)	-	19.6	28.4	29.4	-
Histology					
Normal	3	0	0	1	1
Inflammation	2	1	2	1	2
Hyperplasia	0	0	1	2	2
Carcinoma	0	4	2	1	0

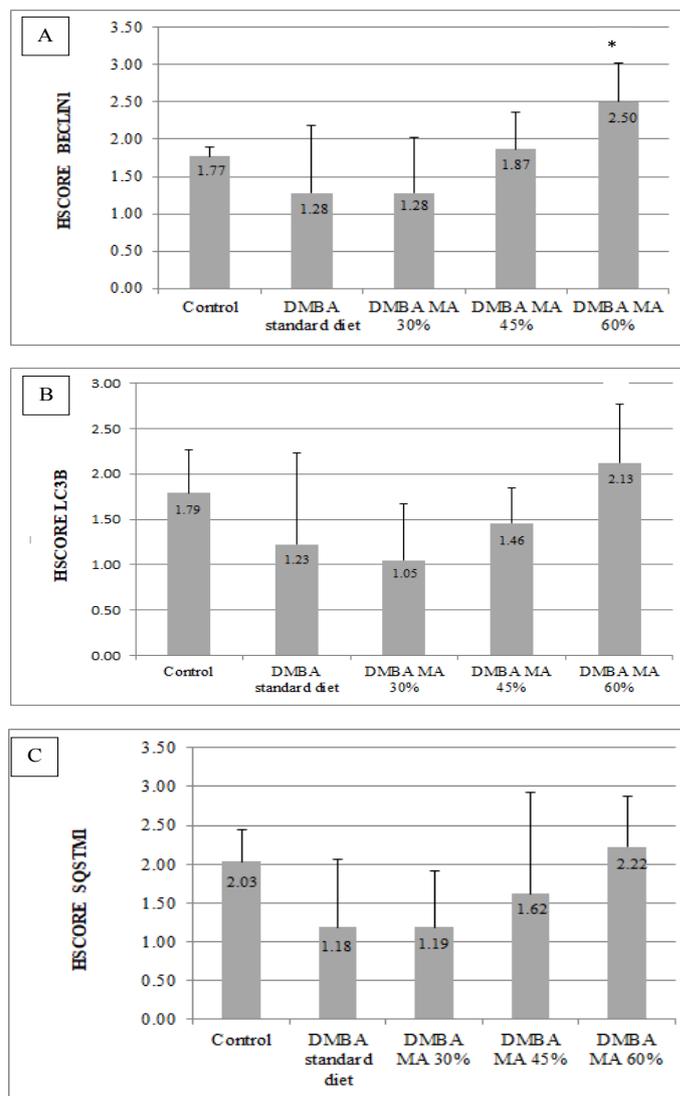


Figure 3. HSCORE of Expression of Autophagy Protein. A. One way ANOVA test of Beclin 1 expression ( $p=0.03$ ) and Tukey's HSD test between 60% MA and DMBA standard diet group ( $p=0.039$ )\*; B. One way ANOVA test of LC3B expression ( $p=0.11$ ); C. One way ANOVA test of SQSTM1 expression ( $p=0.23$ )

and no nodule in 60% MA, respectively. The fastest time of nodule forming was group DMBA standard diet (Table 3). All mammary nodules made histologic preparations. Mammary glands of Sprague Dawley rats that did not experience nodules underwent histologic preparation too.

Histological features of the control group showed normal breast glands: no proliferation, adipose, and connective tissue appeared dominant (Figure 1). One of the nodules of the DMBA standard diet group did not grow cancer. Its mammary glands showed normal with vascular congestion and many polymorphonuclear cells. Four of five nodules of the DMBA-standard diet group showed cancer features indicated by fewer tubules formation, increase in mitosis, the appearance of pleomorphic, and infiltration of surrounding tissue. In the groups of 30% and 45% of MA, there was normal mammary gland with inflammation, mammary gland hyperplasia, and cancer. In a group of 60% MA did not find mammary nodules but, two rats were experiencing proliferation or hyperplasia, one rat with inflammation and two rats with normal breast glands (Table 3).

#### Effect of MA on Autophagy Expressions

Two readers randomly conducted an expression assessment of autophagy protein from 10 slides. Interrater test results showed there was no difference in reading (reliability of alpha and ICC = 0.98,  $p=0.76$ ). Expressions of Beclin 1, LC3B, and SQSTM1 were mostly seen in group DMBA 60% MA followed by the control group and the least were 30% MA and DMBA standard diet (Figures 2 and 3). One way ANOVA tests on the expression of Beclin 1 showed significant differences ( $p=0.003$ ). Post hoc test A Tukey's Honest Significant Difference (Tukey's HSD) showed that Beclin 1 between the 60% MA group and the DMBA standard diet and 30% MA was significantly different ( $p=0.039$ ). On the other hand, there was no difference in Beclin 1 expression between the 60% MA group and the control group ( $p=0.372$ ) and the 45% MA group ( $p=0.513$ ). However, the expression of LC3B ( $p=0.11$ ) and SQSTM1 ( $p=0.225$ ) had no significant difference.

## Discussion

The groups of MA diet ate a decreasing amount of feed compared to standard feed in the early weeks of treatment but their body weight did not have a difference from the standard feed. It means MA administration in the diet does not interfere with the Sprague Dawley rats' growth. MA in this research does not cause a decrease in body weight, because the test animals are in their time of growth. It is because MA contains fiber that causes slowing of digestion and gives more prolonged satiety (Nugraheni et al., 2020). At the last treatment, the bodyweight of the group 60% MA did not differ from the healthy control group, while group 30% and 45% MA started to experience a decrease similar to the DMBA standard diet group. Previous research showed that DMBA administration often causes bodyweight decrease (Kusnul et al., 2019; Rojas-Armas et al., 2020). It indicates that the DMBA induces organ toxicity such as the liver through an oxidative stress mechanism (Arora et al., 2014). DMBA increases the amount of granulocytes and decreases the amount of erythrocytes (Al-Asady et al., 2020). It also increases inflammation (Gasparoto et al., 2014) and declines immunity (Miyata et al., 2001). In groups DMBA 30% and 45% MA, some rats have cancer which causes their appetites to drop so that their body weights decline.

The risk factors of breast cancer can be caused by lifestyle such as dietary habits (World-Cancer-Research Fund, 2017). World Cancer Research Fund Network recommends types of food that contain high fiber and swapping main foods with tubers such as cassava, yam, and potatoes to prevent cancer (WCRF, 2018). MA has the potential in becoming a swapping alternative for the main food because it contains complex carbohydrates with a low glycemic index, but the research of MA toward cancer prevention is still limited (Firoskhan and Muthuswamy, 2021). This research shows that MA dietary can prevent breast cancer. The number of nodules that appeared in the DMBA rat group fed the MA modified diet was less compared to the standard diet. The average weight and volume showed lighter and smaller nodules as well as the time of nodule appearance which was slower. MA contains both soluble and insoluble fiber. A high intake of soluble fiber can increase the production of normal flora and bacteria fermentation so that the production of short-chain fatty acids (SCFA) increases especially butyrate. Butyrate increases the integrity of colon epithelial cells and prevents mutation. Butyrate inhibits the proliferation of colon cancer cells by inducing inhibitors of CDK p21, inhibiting the expression of pro-metastatic genes like MMP. Butyrate can also cause apoptosis of DNA-damaged colonic epithelial cells (Zeng, 2014). MA has potential as an anticancer because consuming MA flour can increase the production of SCFA especially butyrate (Harmayani et al., 2011; Kumalasari et al., 2012). The previous research demonstrated that other types of tubers which also contain high fiber such as *Dioscorea esculantaa*, *Dioscorea opposita*, and *Dioscorea rotundata*, and *Dioscorea zingiberensis* can also inhibit the growth of breast cancer cells (Soetoko and Sumarno, 2012; Chan and Ng, 2013; Zhang et al., 2013).

Autophagy is very crucial for the cell homeostatic process if the cells are stressed. By autophagy, cells can organize damaged organelles as the result of a lesion to be reformed, so that the use of energy is more effective (Karšli-Uzunbas et al., 2014). Autophagy is needed when DNA damage occurs due to chemical aging, ROS, or oncogenesis. In the healing process it stimulates the cells to repair DNA to prevent genome instability (Eliopoulos et al., 2016). When the immune cells of both innate and adaptive do not decrease and can perform their function, it is expected that if cancer occurs, the immune cells can eliminate cancer properly (Zhang et al., 2016). This study showed that the administration of a 60% MA modification in food intake could induce the initiation of autophagy. It is shown from the increased expressions of Beclin1. However, effect of MA on the autophagy process has not been demonstrated as there is no difference in LC3B expression. Beclin1 is the domain of Bcl-2 homolog (BH3) that is an initiator for the autophagy process. Beclin 1 recruits key proteins of autophagy to form a pre-autophagy structure. The average of the Beclin expressions of the DMBA standard diet group had the lowest compared to those given 60% MA in which many breast gland cells were normal. Breast and ovarium cancer can lose 40-70% of Beclin1 causing a decline in the genes expression which is related to a bad prognosis (Marquez and Xu, 2012).

LC3B is one of the main components of the autophagosome membrane and is a marker for autophagy activity (Koukourakis et al., 2015). There is a contradiction about LC3B expression toward cancer. A meta-analysis study showed that increased LC3B expression is related to a bad prognosis of breast cancer (He et al., 2014). Otherwise, administration of mangosteen to DMBA-induced mice can increase LC3B expression and skin cancer inhibition (Wang et al., 2017). In this study, SQSTM1 expression did not change after being given MA. SQSTM1/p62 is an adaptor protein involved in tumorigenesis. P62 that interacts with light chain 3 (LC3) can activate the line of NF-kB transduction signal so that it initiates the avoidance of apoptosis and stimulates carcinogenesis (Katsuragi et al., 2015). High expression of p62 relates to the poor prognosis of patients with triple-negative breast cancer (Luo et al., 2013). However, other researchers showed that p62 is a gene tumor suppressor. Losing p62 expression causes progressivity of prostate cancer (Huang et al., 2018).

MA diet has the potential in inhibiting cancer growth since it contains antioxidants as phytochemical substances (Ruba and Mohan, 2013; Nishaa et al., 2013). Antioxidants such as phenols, flavonoids, and alkaloids are known to have mechanisms that are involved in anti-cancer agency. These phytochemicals have been observed to have a role in inhibiting inflammation since they affect expressions of cytochrome c oxidase subunit 2 (COXII), tumor necrosis factor-alpha (TNF $\alpha$ ), and necrosis factor kappa beta (NFkB). They inhibit the cancer cell growth indicated by the decrease of expressions of EGFR, P13K, cyclin D1, and increased apoptosis (Iqbal et al., 2017). Flavonoids have also been widely studied and can induce autophagy and prevent various diseases. However, the effect of flavonoids plays a different role in various cancer cells as

both inhibitors and inducers of autophagy. In addition, it increases the chemosensitivity of tumor cells and tumor cells' death that are resistant to drugs (Yan et al., 2018; Pang et al., 2021). In conclusion, the present study shows that the MA diet has been proven in reducing mammary cancer risk in rats. Dietary MA acts as an anti-proliferative agent by inducing of Beclin 1. This study highlights the potential of MA as a chemopreventive agent against breast cancer.

## Author Contribution Statement

IF, TA, SW, SH, SS contributed to the planning, research, and writing of this article.

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### General

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### Approval

This research is part of the thesis and had approved by the doctoral program, Faculty of Medicine, Public Health and Nursing, Universitas Gadjah Mada.

### Ethical Declaration

This study was approved by the ethics committee of the Faculty of Medicine, Public Health and Nursing, Universitas Gadjah Mada.

### Conflict of Interest

No conflict of interest.

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