BREAST ANTICANCER ACTIVITY OF BRUCEIN-A FROM MAKASAR FRUIT (*Brucea javanica*) AGAINST EXPRESSION OF GENE p53 IN RAT INDUCED DIMETILBENZAANTRAZENA

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ABSTRACT

Breast cancer is the second leading cause of death after cervical cancer. Treatment of breast cancer is done with radiation, surgery, and chemotherapy which often cause side effects the spread of cancer cells, damaging healthy cells, and mutation. Therefore, it is necessary to find new drugs to treat breast cancer effectively and safely. Previous research showed that brucein-A from makasar fruit (*Brucea javanica*) had breast anticancer activity *in vitro* with IC_{50} 0.54 µg/L significantly different with standard drug of cisplatin (IC_{50} 0.43 µg/L). Encapsulation of brucein-A with liposomes enhance the anticancer activity (IC_{50} 0.39 µg/L). Giving encapsulation of brucein-A dose 10 mg/kg bw did not cause damage to the liver and kidneys of rats with SGPT 21.67 IU/L, SGOT 40.67 IU/L, and reduced breast cancer cells in rats. Therefore, the mechanism brucein-A to treat breast cancer cells need to be further investigated in order to develop brucein-A as drug. This study aims to determine the breast anticancer activity of brucein-A from makasar fruit against expression of gene p53 in rat induced dimetilbenzantrazena (DMBA). This research used 27 female rats which were divided into 9 groups. All groups were given the DMBA orally at a dose of 20 mg/kg bw twice a week for 3 weeks in order to form breast cancer in rat. Brucein-A was given orally to each group with dose 0, 2.5, 5, 7.5, 10, 12.5, 15, 17.5, and 20 mg/kg bw once daily for 7 consecutive days. The rats were then maintained for 28 days and fed *ad libitum*. The results showed that giving brucein-A dose 0, 2.5, 5, 7.5, and 10 mg/kg bw in rats induced DMBA give score 1 of p53 gene expression (<25%) with differentiation degree of good. While, the giving of brucein-A dose 12.5, 15, 17.5, and 20 mg/kg bw showed score 3 of p53 gene expression (>75%) with differentiation degree of moderate to bad.

Keywords: brucein-A, *Brucea javanica*, gene p53, makasar fruit

I. INTRODUCTION

Cancer is a very dangerous disease number two cause of death after cardiovascular. According to WHO, in 2009 an estimated 1.2 million women with breast cancer and more than 700,000 die from the disease. Breast cancer is the second suffered Indonesian women after cervical cancer (Yayasan Kesehatan Payudara Jakarta, 2007). Breast cancer treatment can be done with radiation, surgery, and chemotherapy. However, these medications often cause side effects such as the spread of cancer cells to other parts, damaging healthy cells, and can cause cancer cells to mutate. Therefore, the discovery of new drugs that are effective, safe, and does not cause side effects is needed to treat breast cancer. One alternative is to use brucein-A compound isolated from makasar fruit (*Brucea javanica*).

Makasar fruit is a medicinal plant that is widely used to treat malaria, dysentery, dengue fever, and cancer. Several previous studies have shown that the compounds of quasinoid from these plants have antitumor activity (Lee *et al*, 1984; Fukamiya *et al*, 1992; Rachman *et al*, 2012). Quasinoid compound can induce apoptosis in the degradation of the DNA into the chain oligonukleosom (Subeki *et al*, 2007). Our previous studies showed that the compound brucein-A isolated from makasar fruit showed anticancer activity *in vitro* against breast cancer with IC50 values of 0.54 mg/L was not significantly different from the standard drug of cisplatin having IC50 values of 0.43 mg/L (Ningrum, 2010). Further studies of brucein-A encapsulated with liposomes showed increased anticancer activity with IC50 values of 0.39 mg/L (Subeki *et al*, 2011). Encapsulation brucein-A at a dose of 10 mg/kg bw did not cause damage to the liver and kidneys of rats with ALT levels of 21.67 IU/L, SGOT 40.67 IU/L. Encapsulation of brusein-A at a dose of 10 mg/kg bw can inhibit breast cancer cells in rats (Subeki *et al*, 2013).

*In vitr*o assay of encapsulation of brucein-A have anticancer activity higher than the standard drug of cisplatin. *In vivo* assay of brucein-A can inhibit breast cancer cells in rats, so it needs to be studied further apoptosis mechanism of these compounds. Brucein-A is likely to be cytotoxic against breast cancer growth by inducing the expression of bax and bad, increases the activity of caspase 3, and causing apoptosis (Meergans *et al*, 2000). Brucein-A has the ability to enhance the expression of p53, Bcl-2, and increased the expression of Bax, which in turn induces apoptosis in cancer (Pardhasaradhi *et al*, 2005). Therefore, it is necessary to conduct further research using in vivo animal experiments to determine the mechanism of brucein-A in breast cancer cell. This study aims to determine

the breast anticancer activity of brucein-A from makasar fruit against expression of gene p53 in rat induced dimetilbenzantrazena (DMBA). We hope this research will be known mechanism brucein-A compound in breast cancer cell death and can be made from raw material makasar fruit crops that grow in Indonesia.

II. METHODS

2.1. Place and Time Research

Research was conducted at the Laboratory of Bioactive Components, cage experiments, Laboratory of Medical Education, as well as the Hospital Blood Analysis Laboratory Urip Sumoharjo, Laboratory of Biochemistry, Puspiptek Serpong. The study was conducted in April to September 2015

2.2. Method

This study was conducted in a randomized block design complete with 3 replications. The study was conducted using 27 rats were divided into 9 groups. Each group consisted of three rats. Each group was administered with doses of brucein-A. The data obtained were analyzed descriptive.

2.3. Preparation of Test Compounds

This study begins with the production of brucein-A from *B. javanica* according to the procedure Subeki *et al*, 2007. Amount of *B. javanica* 10 kg soaked in 30 L ethanol solution for 28 days. The filtrate is filtered by the filter cloth and evaporated with a rotary evaporator up to 1 L. The filtrate was concentrated and then extracted with EtOAc to obtain water and EtOAc layers. EtOAc layer was evaporated to dryness and subjected into silica gel chromatography column and eluted with $CHCl_3$ (3 L), MeOH-$CHCl_3$ (3 : 97.3 L), and MeOH-$CHCl_3$ (1: 4.3 L), respectively. Fraction MeOH-$CHCl_3$ (1: 4) was evaporated to dryness and subjected into a silica gel chromatography column and eluted with hexane-EtOAc (1: 1) to be 3 fractions. Fraction 3th was evaporated to dryness and then crystallized with MeOH solvent to obtain compound of brucein-A. Furthermore, to prove that the compound obtained is brucein-A then performed spectroscopic analysis IR, MS, and NMR and compared with the standard brusein- A. Extraction and isolation of brucein-A from *B. javanica* can be seen in figure 1.

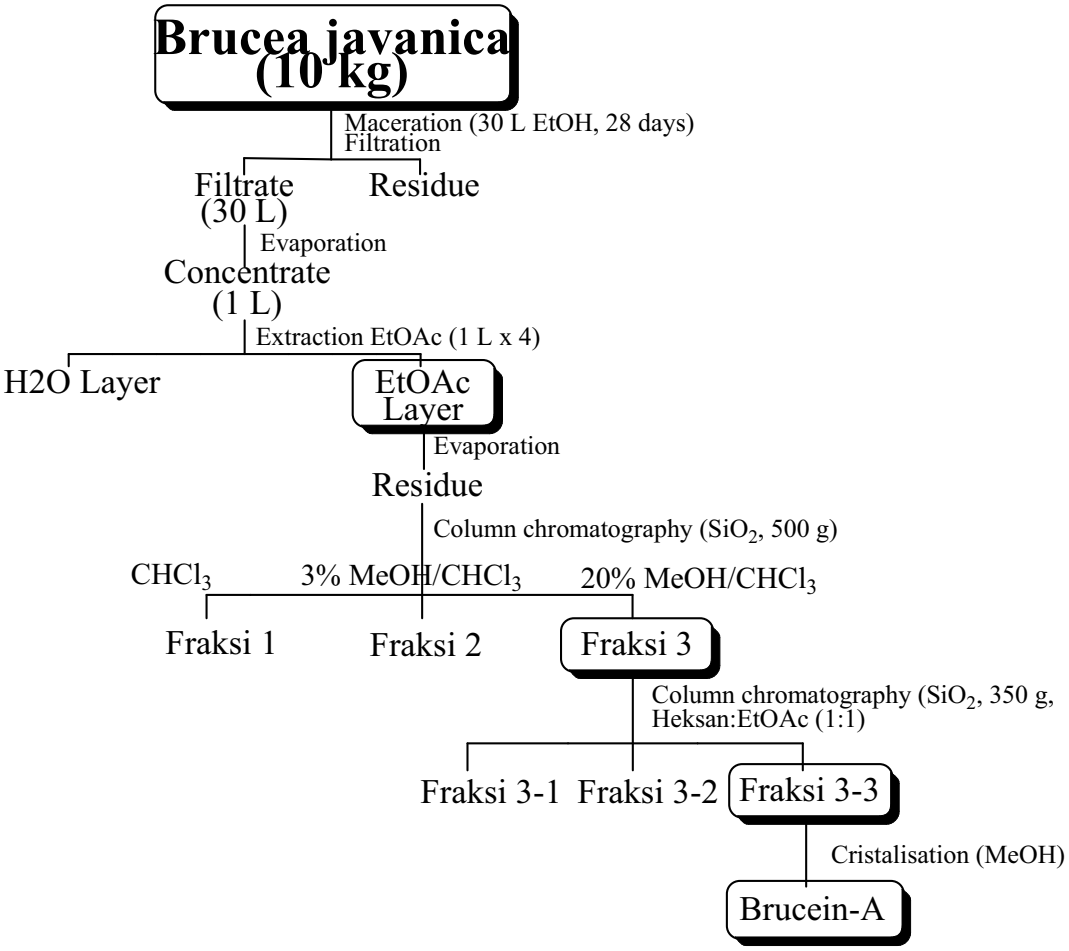

Figure 1. Extraction and isolation of brucein-A from *Brucea javanica*

2. 4. Test Compound Brucein-A against p53 Gene Expression

12 weeks old female rat were divided into 9 groups and each group consisting of 3 rat are placed in separate cages and fed *ad libitum*. Before being treated, rats adapted to the experimental environment for 3 days. All groups of rats were given the compound of DMBA (dimetilbenzantrazena) orally at a dose of 20 mg/kg bw twice a week for 3 weeks in order to form of breast cancer. Brucein-A was given orally to each group of rats with each dose of 0, 2.5, 5.0, 7.5, 10.0, 12.5, 15.0, 17.5, and 20.0 mg/kg bw once daily for 7 consecutive days. One group of rats was used as control without giving brucein-A. The treatments arranged in a completely randomized design with three replications. Furthermore, the rats maintained for 28 days and given feed and drink *ad libitum*. Giving brucein-A in rat induced DMBA can be seen in Figure 2. Expression of p53 is the number of breast cancer

cells that expressed p53 after giving brucein-A by observing the p53 immunohistochemical staining. Score 1 expressed <25%, score of 2 expressed 25-50%, and score of 3 expressed > 75%.

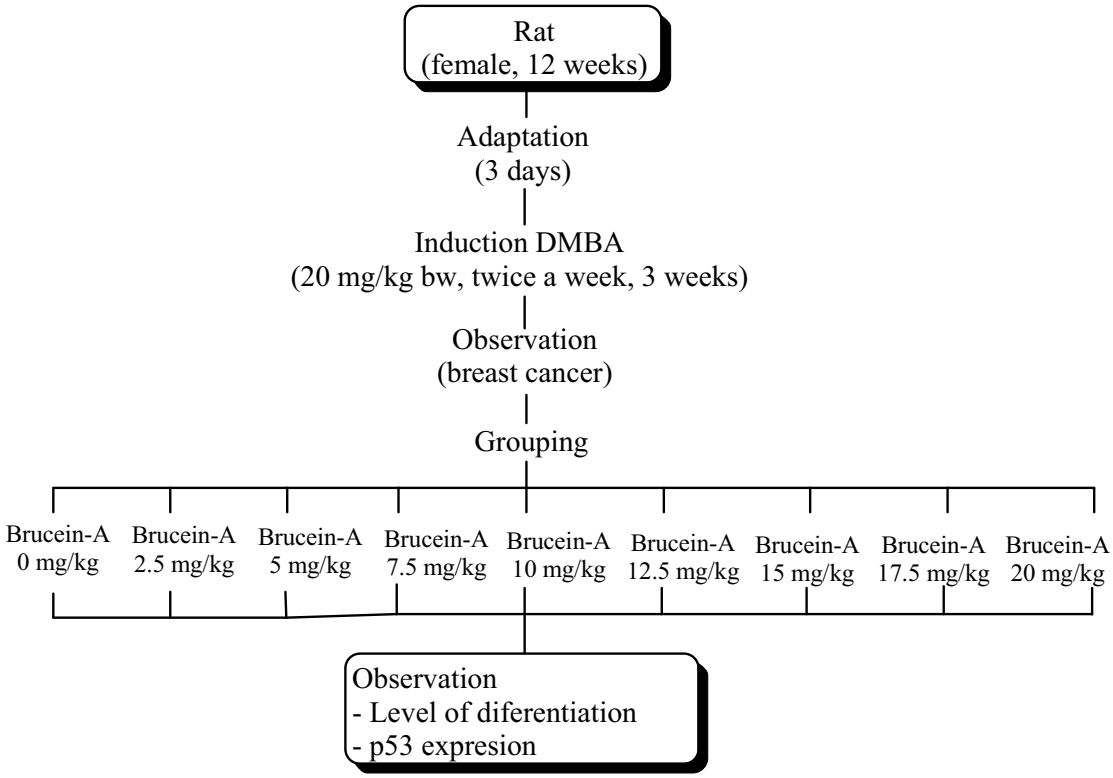

Figure 2. Giving brucein-A in rat induced DMBA

III. RESULTS AND DISCUSSION

3.1 Bruceine-A

A compound of brucein-A was isolated from *B. javanica* having amorphous powder with a melting point of 271-272 °C and optical rotation $[\alpha]^{20}_{D}$ -80.3º (*c* 0.8, piridin). IR analysis showed a hydroxyl group (3420 cm-1), δ-lakton and ester (1736 cm^{-1}), α,β-carbonil (1683 and 1640 cm^{-1}). Results Mass Spectrometer analysis of FD-MS: m/z 522 $[M]^+$ and HR-EI-MS m/z 522.2090 $[M]^+$ which showed the molecular formula $C_{26}H_{34}O_{11}$.

1H-NMR analysis showed proton resonance spectrum of a methyl tertiary (δ 1.22), two secondary methyl (δ 0.90 and 0.91), and a methyl olefinic (δ 1.72). 13C NMR analysis

provides spectrum resonance at C-3 (δ 144.2), C-11 (δ 71.5), and C-12 (δ 74.7) indicating the hydroxy group attached to the carbon. Side chain group containing 3-methylbutanoyloxy related to C-15 is based on the δ 170.0, 42.6, 25.4, 22.3, and 22.4), The chemical structure of brucein-A isolated from *B. javanica* can be seen in Figure 3.

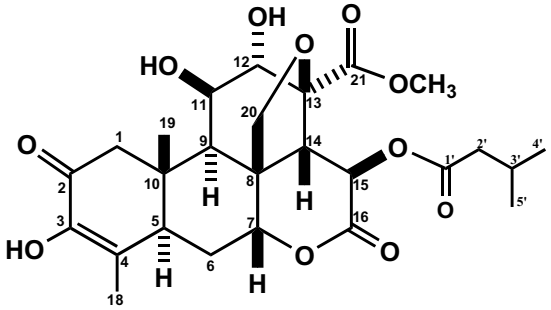

Figure 3. The chemical structure brucein-A from *Brucea javanica*

3.2. Score Cancer Cells that Expressed p53

The cause of breast cancer is still being debated. Some risk factors are thought to be a trigger of breast cancer include genetic factors, age, parity, race, and family history, history of use of therapy or hormonal contraception, and obesity (Granstrom, 2008; Miettinen, 2009; Fauzan, 2009).

Various genetic studies have been developed in order to understand the etiology and pathophysiology of breast cancer, either through direct examination of the gene mutation or indirectly through the abnormality expression of a protein produced by the mutated gene. One of the genes that play a role in the occurrence of breast cancer is p53, the gene which encodes or expresses the protein 53 (p53).

Gene of p53 has a very important role in controlling the cell cycle, apoptosis and maintaining genomic stability. Loss of p53 function as a result of mutations can cause malignant transformation, tumor spread, and tumor resistance to therapy that induces damage of DNA. p53 mutation would produce an abnormal protein with very long half-life that expression can be detected by immunohistochemistry (Choudhury *et al*, 2012).

In this research, immunohistochemical examination of the 9 samples of paraffin blocks of breast cancer. A total of 5 of the 9 samples obtained paraffin blocks were positive p53 expression, in which each of 4 samples with poorly differentiated degrees, 2 samples with the degree of differentiation medium, and 3 samples with a good degree of

differentiation. The occurrence of these variations for the provision of compounds brucein-A in rats could inhibit cancer cell differentiation. The degree of differentiation in breast cancer cells in rats can be seen in Table 1.

Table 1. Scores of breast cancer cells that overexpressed p53 after giving brucein-A with immunohistochemical staining

No	Concentration mg/kg bw	Level cell differentiation	Expression p53
1	0	Good	1
2	2.5	Good	1
3	5.0	Good	1
4	7.5	Moderate	2
5	10.0	Moderate	2
6	12.5	Bad	3
7	15.0	Bad	3
8	17.5	Bad	3
9	20.0	Bad	3

anotation: scor 1 expressed <25%
scor 2 expressed 25–50%
scor 3 expressed >75%

3.3. Breast Cancer Cell Staining

This research with 9 groups of rats suffering from breast cancer used cell staining to identify the prognostic value of p53 expression against breast cancer. The results showed that doses of 0, 2.5, 5.0, 7.5 and 10 mg/kg bw experiencing positive p53 expression with good and moderate degree of differentiation. For a dose of 12.5, 15.0, 17.5, and 20.0 mg/kg bw showed positive p53 expression with degrees differentiate bad. The results of staining of breast cancer cells in rats can be seen in Figure 4.

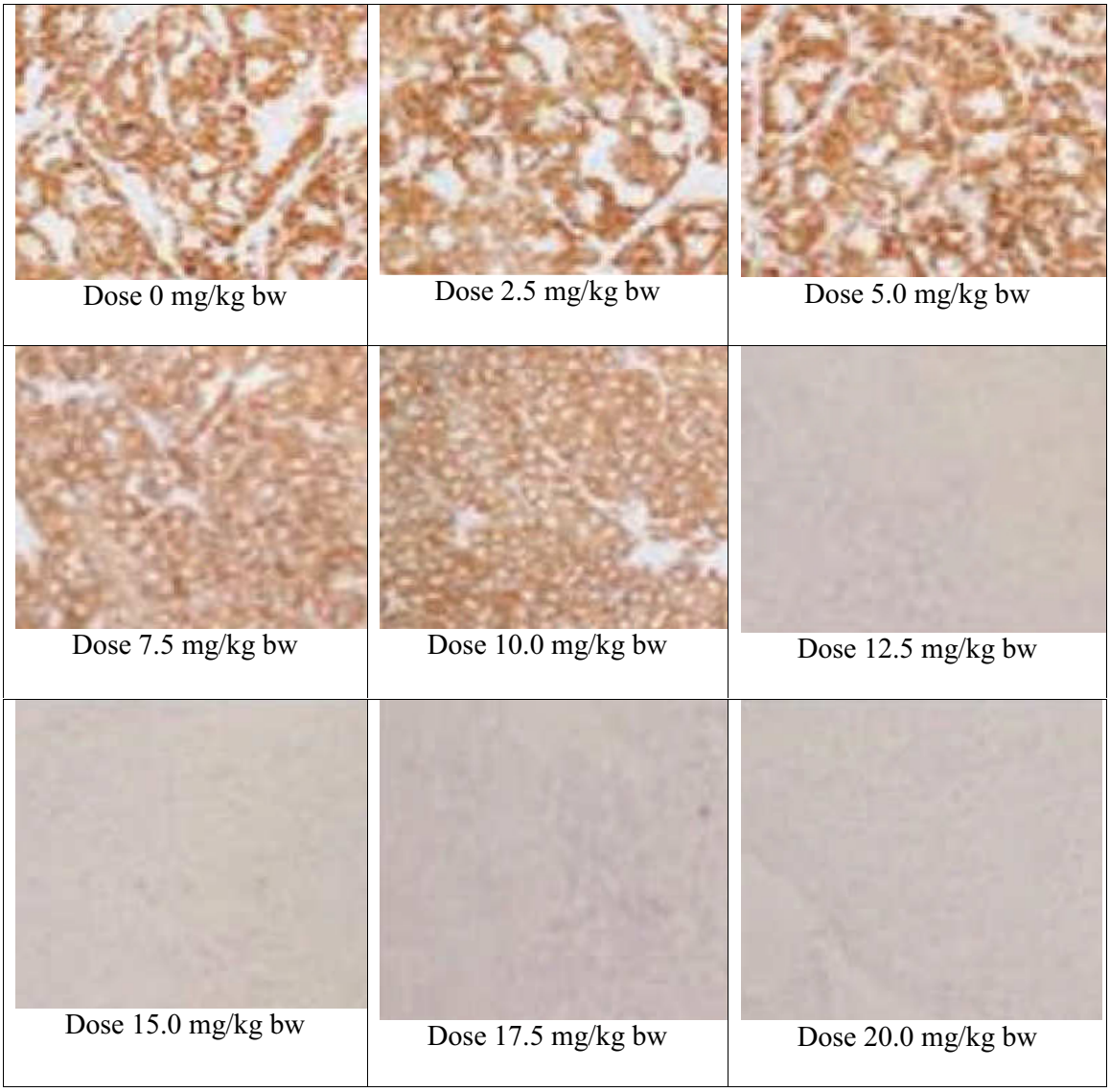

Figure 4. The results of immunohistochemical staining of breast cancer in rats

Graeff (2006) showed that increased p53 expression associated with tumor type serus. Serus type of carcinoma is more common with poor differentiation degree at an advanced stage, while in the mucinous and endometrioid type is more common with a good degree of differentiation at an early stage. Serus type of breast carcinoma more shows the degree of tumor differentiation ugly, while the opposite is the type mucinous and endometrioid more showed good tumor differentiation (Havrilesky, 2013).

Gene expression undergoes many stages ranging from DNA to become proteins. Some studies indicate that the neoplastic cells mutated p53 missense can be observed by immunohistochemical techniques. This is because the p53 mutation produces protein p53 stable and longer half-life. Type p53 mutations frame-shift or nonsense (chain termination/truncated protein) to produce the p53 protein unstable, easily degraded and is not detected by immunohistochemistry (Rauf and Masadah, 2009). This is the reason why the dosage of the compound brucein-A greater than 12.5 mg/kg bw showed p53 expression. These results require further research to identify the type of p53 mutation that occurs in rats as a result of administration of the compound of brucein-A.

Giving brucein-A dose 0, 2.5, 5.0, 7.5, and 10.0 mg/kg bw in rats induced DMBA give score 1 of p53 gene expression (<25%) with differentiation degree of good. While, the giving of brucein-A dose 12.5, 15.0, 17.5, and 20.0 mg/kg bw showed score 3 of p53 gene expression (>75%) with differentiation degree of moderate to bad.

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