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Effect of *Annona Muricata* Leaf Extract on Antioxidant Activity and Histology of the Mamary Tissue in the Breast Cancer model in Vivo

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ABSTRACT

The utilization of medicinal plant for curing some degenerative illness such as breast cancer has gained attention during recent years due to their safety, effectivity and economic reason. However scientific data to prove these habit are still in limitation. This study aimed to determine the effect of ethanol extract of soursop (*Annona muricata* L.) leaves on levels of superoxide dismutase (SOD), Malondialdehyde (MDA) and histological section in the mammary tissue of breast cancer mice models. 20 animal model (mice) induced by 20 mg/kg bw of 7,12-Dimethylbenz (α) Antrasen (DMBA) were treated with extract of soursop leaves in various doses 0 (C-), 100 (T1), 150 (T2) and 200 mg/kg body weight (bw)/day (T3), while for negative control (C-) was only administrated with 0.5% CMC Na/day for 8 weeks and corn oil 2 times a week for 6 weeks. The effect of each treatment was analyzed by one-way ANOVA, and significant difference was analyzed by Duncan's multiple range test. The result revealed that soursop leaves extract with dose of 200 mg/kg bw increased the levels of SOD, and decreased the levels of MDA in mammary tissue and improved the mammary cells of breast cancer mice model close to normal conditions.

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INTRODUCTION

Data obtained from WHO stated that the rate of mortality due to cancer illness has reached 13% or 7.4 million in 2008, and estimated that the death rate will reach 8.1 million by 2020 in the world (Alwan, 2010). Most cases of cancer occur in lung cancer, liver, colon and breast cancer (Ferlay, 2010). In recent years, Cancer drugs often have lethal potency on cancer cells as a whole, which resulted in the emergence of cancer cells that are resistant to cancer drugs. In addition, cancer treatments such as chemotherapy also cause bad side effects for the patient such as weight and hair loss. Side effects arise because cancer drugs used are non-selective, which mean these drugs not only kill cancer cells but also healthy cells. Therefore, it is necessary to find an alternative material that has potential as anti-cancer drugs. One of the plants that have the potential as a cancer drug is soursop plant, which contain active compounds that are cytotoxic and rich in antioxidant (Oberlies, 1997).

Methodology:

Animals used in this study were female mice (*Mus musculus*) balb/c with \pm 40 days old and their

body weight were 18-20 grams. Before used for experimental study, animals were acclimatized for 2 weeks, fed and watered ad libitum. After acclimation, mice were randomly divided into 5 groups and 4 replication. Group 1 was given by 0.5% CMC Na daily for 8 weeks and corn oil 2 times a week for 6 weeks (negative control, C-); Group 2 is given 0.5% CMC Na daily for 8 weeks and induced DMBA 2 times a week for 6 weeks (positive control, C +); where the other groups (Group 3-5) were induced by DMBA 2 times a week for 6 weeks and fed by soursop leaves extract as much as 100 mg/kg bw/day (T1), 150 mg/kg bw/day (T2) and 200 mg/kg bw/day (T3) + 0.5% Na CMC for 8 weeks. The powder of soursop leaves was macerated by using 70% ethanol for 24 hours, and 20 mg/kg of DMBA was homogenized with corn oil at a ratio of 3: 1, a solution of DMBA in every administration has a concentration of 3 mg/ml. Histological section of mammary gland was obtained from 10 weeks-treated mice and observed under Microscop Olympus CX31 by using 400 x magnification in five visual fields. The concentration of SOD and MDA were measured with a spectrophotometer absorbance (500-600 nm). All data obtained from this study were analyzed by

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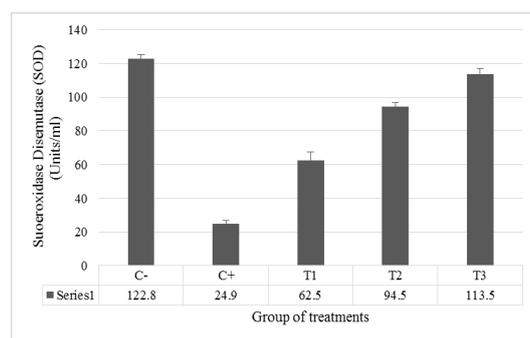
one-way ANOVA and continued with DMRT 1% if value of $F > F(0,01)$.

RRESULTS AND DISCUSSIONS

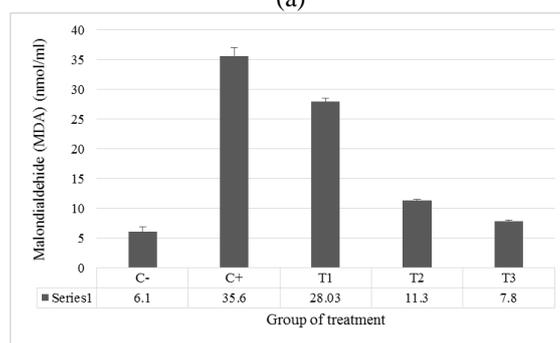
Soursop Leaves Extract Affected SOD and MDA Levels:

Statistical analysis revealed that the ethanol extract of soursop leaves affected the levels of SOD in mammary mice induced by DMBA. The lowest level of SOD was indicated by C+, and the number increased in treatment with T1, T2 and T3, where T3 level was not significantly different with normal (C-). This result suggested that antioxidant activity

increased in breast cancer model which was administrated with ethanol extract of soursop leaves (Fig.1.a). SOD enzyme plays an important role as an endogenous antioxidant protecting cell damage. Based on the mechanism, this enzyme is classified as primary antioxidants which act to reduce the formation of new free radicals by breaking the chain reaction and turning it into a more stable product. The treatment can increase the activity of intracellular antioxidant enzymes. The increasing of antioxidant enzyme activity is associated with decreasing the levels of MDA. The vulnerability of a tissue against oxidative damage depends on defences mechanisms of intracellular antioxidant enzyme.



(a)



(b)

Fig. 1: (a) Level of SOD and (b) MDA of mammary after treatment of soursop leaves extract.

Result showed that the ethanol extract of soursop leaves affected the levels of MDA in mammary mice induced by DMBA (Fig.1.b). The highest level of MDA was demonstrated by C+ and then decreased in the T1, T2 and T3, while the lowest score was observed in normal mice. This result suggests that ethanol extract of soursop leaves decreased lipid peroxidation by oxidation production of unsaturated fatty acid generated through the oxidation by free radical compounds. This was presumably because soursop leaf contained several important compounds as antioxidants, especially flavonoids. Soursop leaf also contain several active ingredients such as annonains, saponins, and tannins (Torres, 2012). MDA was formed from unsaturated fatty acids undergo a process of lipid peroxidation which later became decomposed. MDA was formed relatively

constant so that it was a good indicator to know the presence of lipid peroxidation.

Soursop Leaf Extract Affected Mammary Histological Section in Breast Cancer Model:

Soursop leaf extract significantly reduced the hyperplasia of ductal and alveolar epithelial cells, then dilated the lumen diameter of ductal and alveolar in the mammary tissue of mice. Normal mice (C-) only has two layers of epithelial cells and lumen diameter look likes wider than others (Fig.2). Geneser (1993) stated that lactiferous ductal has two epithelial layers, a cuboid basal cells and a columnar surface cells. The proliferation of ductal and alveolar epithelial cells of mice increased sharply, so that lumen diameter looked very narrow (C+). Hyperplasia epithelial cells and narrowing of the lumen are still observed in T1 and this condition has

decreased in T2 and the condition was close to normal in T3 (Figure 2 and 3). Hyperplasia of epithelial cell in mammary ductus and alveolie decreased sharply on T3. This is caused by soursop

leaves extract that contain 18 types of annonaceous acetogenin. Annonaceous acetogenin has been shown to be cytotoxic in vitro, and its ability for cytotoxic level was 10,000 times stronger than chemotherapy.

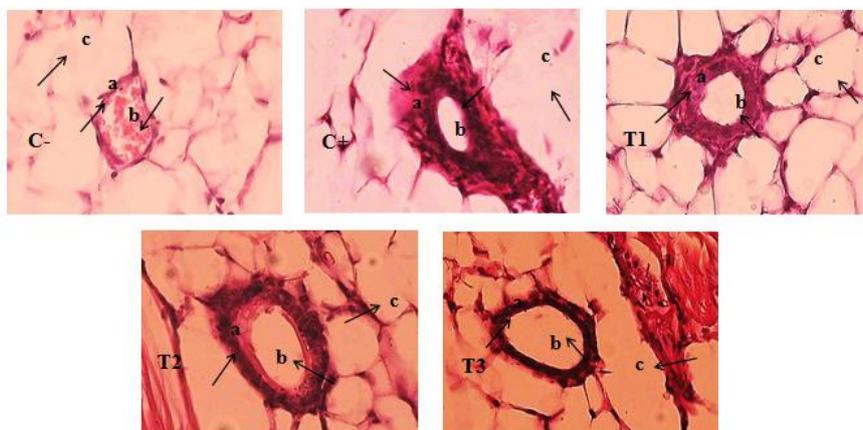


Figure 2, The epithelial tickness and lumen diameter of duktus mamary in all treatments. (a) duktus epithel, (b) lumen duktus and (c) fatty cells (magnification = 400 X)

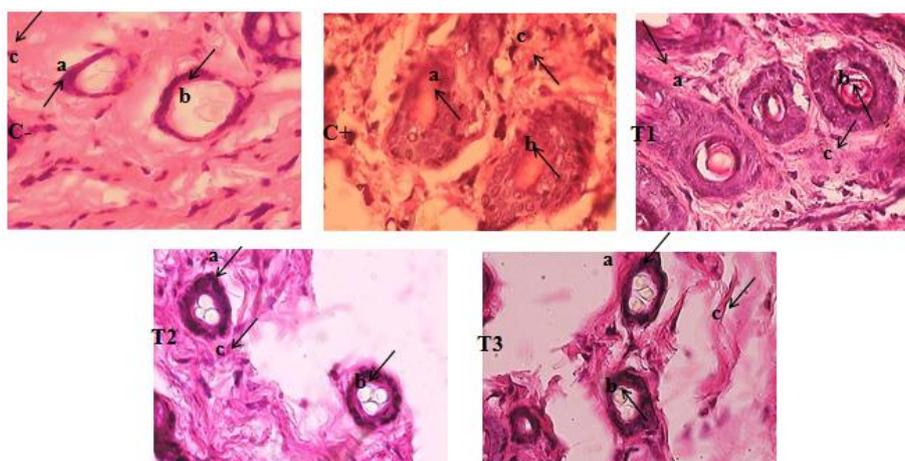


Fig. 3: The epithelial tickness and lumen diameter of alveole mamary (a) alveole epithel, (b) lumen alveole and (c) connective tissue ((magnification = 400 X)).

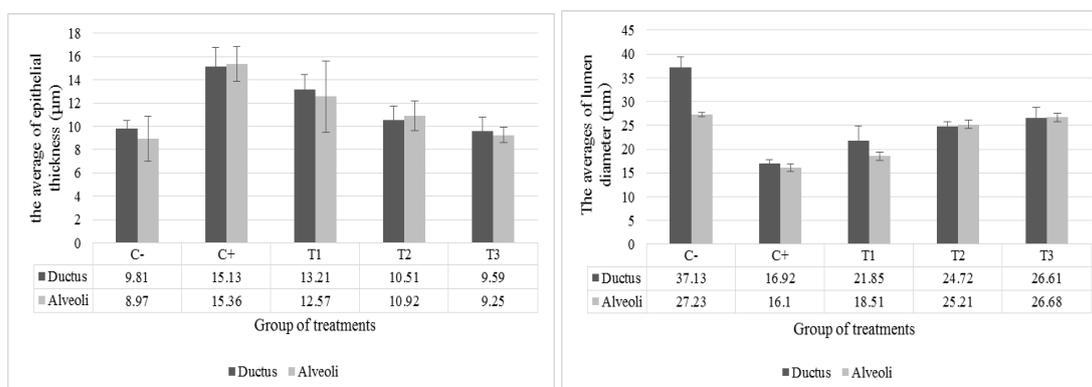


Fig. 4: (a) The averages of epithelial thickness of ductus and alveole (b) lumen diameter of ductus and alveole of mamary after treatment of soursop leaf extract.

Acetogenins are also powerful inhibitors of mitochondrial complex I or NADH dehydrogenase that could decrease ATP production which caused the death of cancer cells. The mechanism of inhibition is optimized by the acetogenin through activating apoptotic pathways and tumour suppressor genes p53, that finally could stop cell cycle and prevent uncontrolled proliferation. Tumour suppressor gene is required to maintain cell division under control. When tumour suppressor genes are not properly functioning, cell proliferation cannot be controlled and lead to cancer (Vousden, 2002; Ryan, 2001). Mutations in the p53 gene is the most frequent genetic mutations found in cancer cells (Ryan, 2001). Soursop leaves contain flavonoids that have a very important role in inhibiting the proliferation of cancer cells. Flavonoids generally have antioxidant activity due have a phenolic hydroxyl groups that can capture free radicals, species that perform oxidation reactions in the cell. With these antioxidant properties, flavonoids could inhibit the initiation of carcinogenesis process by inhibiting carcinogens activity and improving the tissue through cell regeneration (Thierry Soussi and Christophe Beroud, 2001).

Summary:

The result of this study revealed that there is significant effect of ethanol extract of soursop leaves with various doses on SOD, MDA, and the histological section of mammary tissue of breast cancer mice model. Treatment of 200 mg/kgBW significantly decreased hyperplasia of mammary epithelial cells.

It is advisable to do further research about the side effect of ethanol extract of soursop leaves

against the enzyme and histology of hepar to ensure detoxification by this organ.

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