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Research paper



Extract of Calotropis gigantea Leaves to Repair the Histological Profile of Fibrosarcoma Mice (Mus musculus)

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Abstract

Calotropis gigantea (C. gigantea) is widely known as a traditional medicine for diseases such as toothache, colds, asthma. It also used be as a detoxifying agent and an anticancer drug. The leaves contain cardenolide, a bioactive agent which has cytotoxic properties against cancer cells, particularly fibrosarcoma. The active ingredient is able to control the mitochondria (the energy-producing cell organelle) essential for the growth of cancer cells. This study aimed to determine the effect of ethanol extract of C. gigantea leaves on the histological profile of fibrosarcoma mice (Mus musculus). The mice was induced with 7,12-Dimethylbenz [a] anthracene (DMBA) 25 mg/kg bw twice for 6 weeks, then treated by extract of C. gigantea leaves for 2 weeks. This experimental study employed a completely randomized design (CRD) with 6 treatments and 5 replications. The treatments consisted of negative control (C-), positive control (C+), dose of 50 mg/kg bw (T1), dose of 100 mg/kg bw (T2), dose of 150 mg/kg bw (T3), methotrexate dose of 2.5 mg/kg bw (T4). The study observed 30 male mice aged 50-60 days. The data consisted of the number of fibroblasts and the level of collagen fiber damage in skin tissue. The data were analyzed by one way ANOVA, followed by DMRT 1 %. The results showed that the extract of C. gigantea leaves repaired the histological profile of fibrosarcoma mice significantly, indicated by the decreasing of fibroblast proliferation and repairing of collagen fibers in mice skin tissue. The extract of C. gigantea leaves at a dose of 150 mg/kg bw (T3) gave a better effect to repair the histological profile of fibrosarcoma mice than the cancer drug methotrexate synthesis dose of 2.5 mg/kg bw (T4) and other treatments.
Keywords: Calotropis gigantea, fibrosarcoma, DMBA

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1. Introduction

According to WHO, in 2010 the number of cancer patients in the world is about 7 million people. The empirical data shows that the deaths due to cancer increase every year. The results of Basic Health Research in 2007 indicated that about 5.7% of them was caused by malignant cancer (Jemal et al., 2011).

Fibrosarcoma is a cancer derived from mesenchymal cells, dominated by fibroblast cells. It often occurs in the muscles, joints, fat, nerves, skin tissue and blood vessels (Abilash, et al., 2013). National Center for Health Statistics (NCHS) in Indonesia estimates that there were around 7.1% cases of fibrosarcoma of all diagnoses sarcoma in 2010 and with mortality rate of 3.7 % (Jemal et al., 2011).

In the last two decades, treatment of sarcoma patients in Indonesia consisted of surgical as well as treatment of a combination of chemotherapy and radiotherapy in avoid amputation (Bramwell, 2003). All of these treatments have strong side effects. Not only kills cancer cells, chemotherapy treatment also attacks normal cells, especially cells that divide rapidly. Another problem in cancer treatment is the high cost of the treatment (Nair et al., 2013).

One method of cancer treatments could be the use of anticancer agents from natural materials. It might be more secure because could have potential benefits of treatment by natural compound compared with surgery and radiotherapy. It is able to treat the disease by repairing the damaged cells, tissues and organs by boosting the immune system (Bhanot et al., 2011).

The ethanol extract of C. gigantea leaves has antitumor activity against Ehrlich's ascites carcinoma (EAC), when mice were administered intraperitoneally at doses of 50, 100 and 200 mg/kg bw. The dose used in this study is equivalent to the dose of earlier study. It is known that more than 23 kinds of bioactive compounds were found from this plant. The compounds acted as an anticancer agent are alkaloids and cardenolides. Cardenolide is a derivative of terpenoid compounds that is very useful in attacking cancer cells. It doesn't attack normal cells, because it acts in a high specific way for cancer cells (Kumar et al., 2013).

Cardenolide can control the mitochondria. Mitochondria generate the energy in the form of ATP (Adenin Triphosphate) needed for the growth of cancer cells. Cardenolide is a specific inhibitor of the ATPase enzyme, which causes the decreasing of ATP production and energy shortages then induces the death of cancer cells (Agrawal et al., 2011). This study used ethanol as extraction solvent, because terpenoid compounds (cardenolides) are polar and they dissolve well in polar solvents (Wink, 2015).

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Based on this background, this research was expected to provide benefits and scientific information regarding the ability of C. gigantea leaves in inhibiting the growth of cancer cell, particularly fibrosarcoma.

2. Material and Methods

This study employed an experimental design using a completely randomized design (CRD) with 5 treatments and 5 replicates. This study determined the influence of ethanol extract of C. gigantea leaves in different concentrations on the fibrosarcoma histology of mice skin tissue induced by 7,12-dimetilbenz (α) anthracene (DMBA) at dose 25 mg/kg bw twice a week as long as 6 weeks, then treated by extract of C. gigantea leaves as long as 2 weeks. The treatments were negative control or normal mice (C-), positive control or fibrosarcoma mice without treatment of C. gigantea extract (C+), treatment of C. gigantea extract at dose of 50 mg/kg bw (T1), dose of 100 mg/kg bw (T2) and dose of 150 mg/kg bw (T3), methotrexate at dose of 2.5 mg/kg bw (T4). Animal model of balb/c male mice aged 50-60 days were acclimatized for 7 days.

2.1. Extraction of C. gigantea leaves

The procedure for the extraction of C. gigantea leaves referred to the procedure previously described by Muchtaromah et al.. (2011 and 2016) on Centella asiatica leaves.

2.2. Preparations of 7,12-Dimetilbenz (α) anthracene (DMBA)

Provision of DMBA was given at a concentration of 25 mg/kg bw. The solution was the homogenized with acetone. A total of 0.1 mL DMBA was prepared by dissolving 25 mg DMBA in 0.1 ml acetone. Induction of DMBA for each mouse was done twice a week, on Monday and Thursday.

2.3 Data Collection and Analysis

A surgery was performed after 8 weeks of treatment. The mice were sacrified. The samples were taken from fibrous tissue of subcutaneous area for making histology preparation. The number of fibroblast cells were counted from 5 field of view randomly for each mouse, then the average score was taken. The percentage of collagen fiber damage was shown by the width of empty space at skin tissue using millimeter block. The empty space showed the decreasing numbers of collagen fiber.

The data were analyzed using one-way ANOVA and DMRT (Duncan Multiple Range Test).

3. Result and Discussion

The effect of C. gigantea on the number of fibroblast cells of skin tissue

The results showed that the extract of C. gigantea leaves reduced the number of mice fibroblast cells. The data found that the highest number of fibroblast cells at fibrosarcoma mice C+ was 309.56, then the number of fibroblasts decrease in T1 (249.48), T2 (119.00), T4 (97.03), T3 (92.84) and the lowest was found in normal mice C- (51.48). It indicated that it is possible to prevent proliferation of fibroblast cells using extract of C. gigantea leaves.

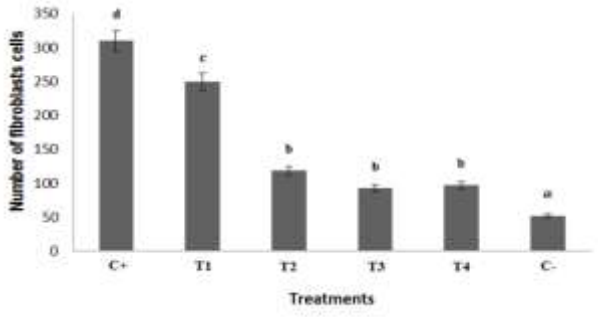


Fig 1: The average number of fibroblasts cells in the treatment of *C. gigantea* extract (C-) Negative Control, (C+) Positive Control, (T1) 50 mg/kg bw, (T2) 100 mg/kg bw, (T3) 150 mg/kg bw, (T4) methotrexate 2.5 mg/kg bw.

Figure 1. showed that the lowest number of fibroblast cells was found in normal mice (C-) and the highest number found in fibrosarcoma mice (C+). The dose of C. gigantea extract that could reduce the proliferation of fibroblasts cells was at dose of 150 mg/kg bw (T3). It was not significantly different with that of 100 mg/kg bw (T2) and 2.5 mg methotrexate mg/kg bw (T4). The T3 and T2 were equivalent to the standard drug of methotrexate (T4), but they were significantly different with other treatments (T1, C- and C+).

The cardenolide in C. gigantea extract might play an important role for inhibiting cell proliferation because of its specific function in mitochondria controlling. Mitochondria were the energy-producing organelles such as adenine triphosphate (ATP) needed for the growth of cancer cells. As a specific inhibitor of the ATPase enzyme, cardenolide decreases the production of ATP and causes energy shortages in cancer cells. The condition leads to the death of the cancer cells and can be controlled (Seeka & Sutthivaiyakit, 2010).

3.1. The effect of C. gigantea on the level of collagen fiber damage in skin tissue

Figure 2 showed that the lowest level of collagen damage was found in normal mice (0.69%) followed by C. gigantea extract at dose of 150 mg/kg bw (5.53%), dose of methotrexate 2.5 mg/kg bw (7.15%); C. gigantea extract at dose of 100 mg/kg bw (13.72%); C. gigantea extract at dose of 50 mg/kg bw (18.16%). The highest level of collagen damage was in fibrosarcoma mice without the treatment of C. gigantea extract (23.71%). These mean that group T3 (dose of 150 mg/kg bw) had the ability to repair the collagen fibers better than the standard drug methotrexate. While the dose of methotrexate 2.5 mg/kg bw (T4) was not significantly different with the dose of 150 mg/kg bw (T3). The dose of 150 mg/kg bw (T3) was equivalent to the cancer standard drug methotrexate, but it was significantly different with other treatments (C-, T2, T1 and C+).

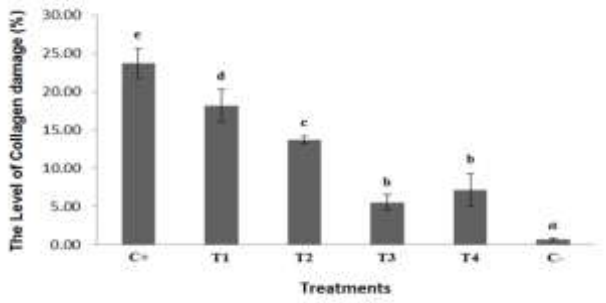


Fig 2: The average level of collagen fiber damage in the treatment of C. gigantea extract. (C-) Negative Control, (C+) Positive Control, (T1) 50 mg/kg bw, (T2) 100 mg/kg bw, (T3) 150 mg/kg bw, (T4) methotrexate 2.5 mg/kg bw.

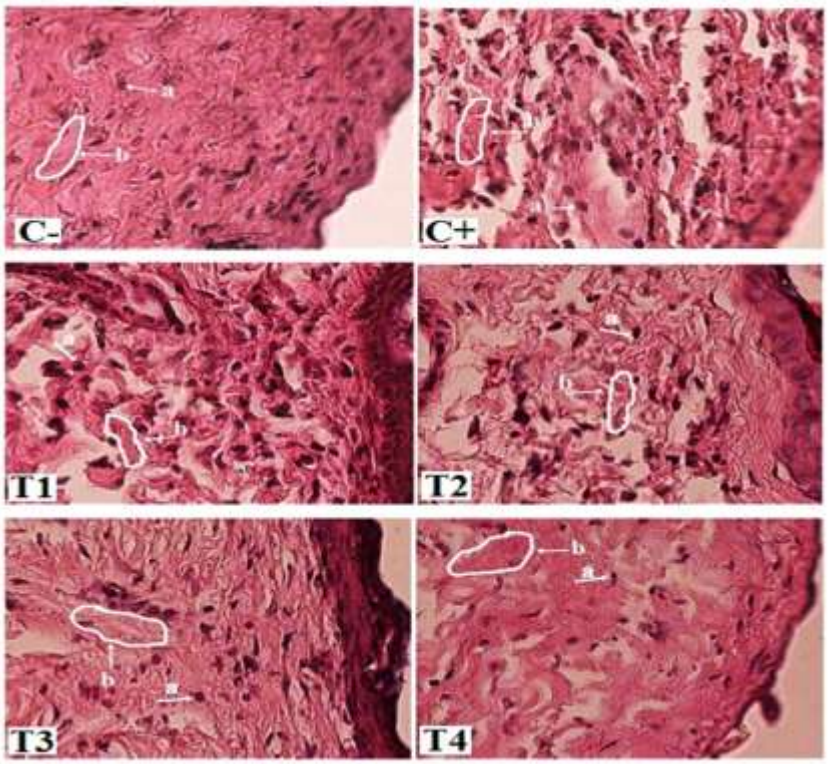


Fig 3: Histological profile of mice skin tissue (400x). (C-) Negative Control, (C+) Positive Control, (T1) 50 mg/kg bw, (T2) 100 mg/kg bw, (T3) 150 mg/kg bw, (T4) methotrexate 2.5 mg/kg bw. (a) Fibroblast Cell, (b) Collagen fibers.

Figure 3 described the histological profile of the level of collagen fiber damage. The skin tissue of normal mice (C-) had many compact tissues consisting of fibroblasts and collagen fibers. According to Agrawal et al. (2011), the normal fibroblast cells have a thin shape with small nuclei surrounded by collagen fibers. The fibrosarcoma mice (C+) without C. gigantea treatment had a wide tissue damage, amounted to 23.71%. The empty spaces showed the decreasing collagen fibers and fibroblast cells. The treatment groups (T1, T2 and T3) showed the repairing of collagen fiber and fibroblast cells in the skin tissue was almost the same with T4 (methotrexate). Phytochemical content in the leaves of C. gigantea such as phenolic acids, polyphenols, flavonoids, flavonols, terpenoids, vitamin C, vitamin E, carotenoids, phenolic acids, phytate, and phytoestrogens were believed to play an important role in suppressing the activity of free radicals thus inhibiting the mechanism of cell damage (Seeka & Sutthivaiyakit, 2010).

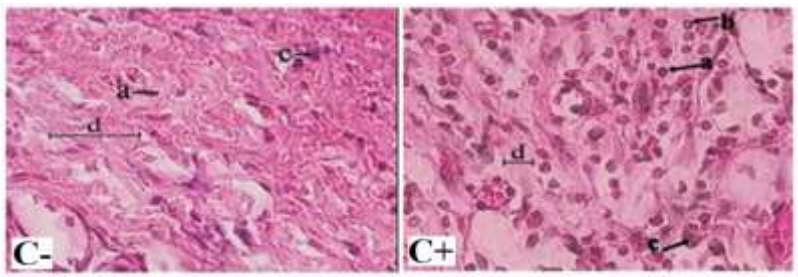


Fig 4: Histological profile of normal (C-) and fibrosarcoma (C+) mice skin tissue (400x). (a) Pyknosis, (b) Karyorrhexis, (c) The mitosis cell, (d) Collagen Fibers

Figure 4. showed that histological tissue of fibrosarcoma mice (C+) did not only indicate the presence of fibroblast cell proliferation, but also showed the stages of cell necrosis.The cells underwent necrosis stages such as pyknosis and karyorrhexis. Necrosis cell death occurred unnaturally. The cell necrosis brought effects such as the cell would swell and it became damaged. The damaged cell could not be destroyed by phagocytes, which lead to the damage of neighboring cells (inflammatory). An apoptosis was a programmed cell damage so its cell size did not change. Since the damaged cells were directly ingested by phagocytes, it did not disturb or damage the neighboring cells (Noolvi et al., 2011). It also showed a proliferation, cells which underwent a mitosis. This indicated the proliferation of fibroblasts cells and necrosis cells. There were a lot of cells with different size of nucleus (pleomorphism). Pleomorphism was a characteristic of fibrosarcoma. On fibrosarcoma, cell nucleus also experienced hypercromatin, i.e cell nuclei which was darker and denser. In contrast with (C-) groups, they had more homogeneous cell nuclei and no visible necrosis.

Cell abnormalities are usually characterized by necrosis. The cells undergoing necrosis will release their contents and become empty cells. The process can occur to respond a specific stimuli, for example an oxidative stress. It is a balance disturbance between the oxidants and antioxidants that causes potential cell damage (Wink, 2015). Caruso et al., (2011) states that the necrosis consists of several steps such as pyknosis (the cell nuclei shrink or wrinkle), karyorrhexis (the core is crushed to form fragments of chromatin materials scattered in the cell), and karyolysis (the core of dead cells cannot be colored any longer).

4. Conclusion

Based on the results above, it can be concluded that there are significant effect of C. gigantea extract on fibrosarcoma histology of mice induced by DMBA in vivo. An obtained optimal dose of extract in treating cancer fibrosarcoma is a dose of 150 mg/kg. The study suggests further research to determine the effect of ethanol extracts of C. gigantea and DMBA on the shape and size of fibroblast cells.

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