The Alkaloid Fraction Of Achyranthes Aspera Linn Increase NK Cells in Breast Cancer Cells

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ABSTRACT

Achyranthes aspera Linn leaf is used to treat cancer, particularly breast and cervix cancer. The alkaloid fraction of leaves Achyranthes aspera linn cause of death in accordance with the mechanism of myeloma cell apoptosis and necrosis associated with p53 gene and cyclin-dependent kinase4. We induced mammary cancer using benzo(a)pyrene among the mice being studied to elucidate activity of leaf extracts of Achyranthes aspera linn to cure mammary cancer. The Mice with mammary cancer were divided into five groups such as mice which were orally treated with alkaloid Achyranthes aspera linn of 0 mg/kg, 10 mg/kg, 30 mg/kg, and 100 mg/kg and the other group were treated with standard anticancer drug cyclophosphamide 6 mg/kg for positive control. We also used healthy mice treated with Olifarum as a negative control. After eight weeks of treatment, mice were sacrificed to evaluate the amount of NK Cells in mammary tissues. The results show that Achyranthes aspera Linn alkaloids increased the number of NK cells in breast cancer tissues. Moreover, the better activity of Achyranthes aspera linn is seen when the dosage is 30 mg/kg bw until 100 mg/kg bw is shown to have a similar effect with Cyclophosphamide 6 mg/kg bw.

KEYWORDS: NK cell, alkaloid, Achyranthes aspera, Breast cancer

INTRODUCTION

Cancer, an abnormal tissue mass grows tremendously in non-coordinated fashion and this growth continues even though the stimulus is lost. It is the second highest cause of death in the United States, after heart disease. The mortality rate due to cancer in the United States is more than 500,000 people each year, of which 50% are affected by cancers that affect breast, lung, prostate, colon and rectum. Breast cancer is the commonly found cancer all over the world with a relatively high incidence of about 20% which is highest among all malignancies [12].

Early stage breast cancer treatments assure recovery up to 75%. Cancer can be treated through various ways including surgery, radiation, chemotherapy, immunotherapy and endocrine therapy. With current treatment methods, one-third of cancer patients can be treated with surgery or radiation therapy, which is quite effective if it has not yet developed metastases [3]. Micrometastasis condition required chemotherapy to kill the primary neoplasm and hidden micrometastasis cells before it gets spread and it can be detected by physical examination or x-rays and the success of breast cancer treatment can be measured after 5-years. The factors affecting prognosis and survival of breast cancer patients are large tumor, status of regional lymph nodes, swelling of skin, menopausal status, tumor cell growth, presence of residual tumor, type of pathology and metastasis therapy, estrogen receptor, age, and large breasts [4].

The research to find new drugs for cancer that selectively kills cancer cells without harming normal cells is an need of the hour task to be completed at the earliest. One of the anticancer drugs derived from plants that
warrant anticancer properties is Achyranthes aspera Linn. The methanol extract of Achyranthes aspera Linn possess antimitotic effects that inhibit the cleavage cells [16,1] and reduce spermatogenesis [10]. Achyranthes aspera Linn contains saponins, alkaloids, flavonoid, betaine, akirantin, rhamnose, glucose and galactose. The researchers also found that flavonoid compounds present in this species are α-spinasterol, β-sitosterol, chrysophanol, dibutyl phthalate, palmitate, α-spinasterol-3-β-d glycoside and daucosterol dan ecdysonerone (Gao et al, 2000). It also contains flavonoid ecdysonerone and alkaid betain. The aim of the study is to evaluate the effect of Achyranthes aspera Linn toward NK cells.

MATERIAL AND METHODS

A. Plant Collection and Preparation of the Extract:

Fresh Jarong leaf samples of Achyranthes aspera Linn. were collected from Surabaya, East Java. This study uses alkaidal fraction. Fractionation of Achyranthes aspera was done at Pharmacy Laboratory, Airlangga University, Surabaya, East Java. The alkaidal fraction was produced on the basis of Pharmacopeia Indonesia method and the alkaid was measured using HPLC, after analysis using TLC. In fractionation, solid phase silica gel is used in which the mobile phase ratio is ethyl acetate: methanol: water = 7:4:1 [5].

B. Mice model development and treatment:

Female Swiss Webster mice (Mus musculus) weighing 20-40g, age two months were obtained from Central Veterinary Pharma, Surabaya, East Java and were housed under hygiene conditions (temperature 22-25°C and humidity 70-80%) for two weeks for acclimatization. Mice (n=60) were divided into 6 groups (of n=10 in each group) and water was given ad libitum prior to the experiment. The animals were treated as follows:

C. Treatment:

Group I served as negative control and mice in group I were treated with Oilmolizarum dose 0.5 cc by injecting subcutaneously at 4 location posterior around rat mammae. The mice groups II-VI were developed as mammary cancer model by injecting benzo(α)pyrene at 10 mg kg⁻¹ dosage subcutaneously around the mammary glands for 8 weeks with injection for 3-day intervals. The mammary cancer in mice were examined through macroscopic monitoring on development of a lump followed by histopathological analysis. 50 mice with mammary cancer were divided into five groups such as those mice which were treated with oral alkaidal Achyranthesaspera Linn everyday with CMC 0,5% as group II, 10 mg kg⁻¹ as group III, 30 mg kg⁻¹ as group IV and 100 mg kg⁻¹ as group V whereas the group VI were treated with a standard anticancer drug named cyclophosphamide 6 mg/kg which served as positive control also.

All groups were treated for eight weeks and kept under observation with intervals of three days. Necropsy was done after all the treatments were completed and preparations were done for immunohistochemistry. Counting transactions are carried out with 100x and 400x magnification microscope

D. Statistical Evaluation:

The groups were compared using one-way analysis of variance (One-way ANOVA) (followed by Tukey test and P<0.05) which was considered significant. The data was analyzed as average from five repetition ± standard deviation.

Result:

The effect of alkaidal fraction of the extract from leaves of Achyranthes aspera Linn toward NK cell on breast cancer cell was significant and dose-dependent (Table 1). From the table 1, it can be seen that the increasing dose administration of the alkaidal fraction increases the amount of NK cells. NK cells serve as cytotoxic cells toward breast cancer cell in mice. ANOVA result shows a significant difference based on the difference found in the dose administration of the alkaidal fraction of the Achyranthes aspera Linn leaves extract, positive and negative control. The administration of alkaidal fraction of leaves of Achyranthes aspera Linn at a concentration of 30 mg kg⁻¹ provides the same effect when using cyclophosphamide 6 mg kg⁻¹ in vivo, at concentrations of 100 mg kg⁻¹ give better effect (6.77 ± 0.42) than positive control (6.37 ± 6.40).

Table 1: NK cell Achyranthes aspera Linn leaves extract

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Dose</th>
<th>NK cell</th>
</tr>
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<tbody>
<tr>
<td>Control negative (Oilmolizarum)</td>
<td></td>
<td>7.53a±0.23</td>
</tr>
<tr>
<td>Control Positive (Cyclophosphamide) 6 mg kg⁻¹</td>
<td></td>
<td>6.37c±6.40</td>
</tr>
<tr>
<td>P1 ( CMC 0.5%)</td>
<td></td>
<td>3.90e±0.22</td>
</tr>
<tr>
<td>P2 (10 mg kg⁻¹)</td>
<td></td>
<td>5.80d±0.28</td>
</tr>
<tr>
<td>P3 (30 mg kg⁻¹)</td>
<td></td>
<td>6.47bc±0.42</td>
</tr>
<tr>
<td>P4 (100 mg kg⁻¹)</td>
<td></td>
<td>6.77b±0.42</td>
</tr>
</tbody>
</table>

*) Values are mean ±standard deviation(n=10) **) Statistically significant difference in respect to the control P<0.05 (BNT 5 %)
Discussion:

The process of cell death through apoptosis in NK cells activates the caspase through perforin and granzyme B. The mechanism of perforin facilitates the transmitting granzyme B into target cells is not clear since it does not require a plasma membrane pore formation [11]. Granzyme B, the prototype of serine proteases, promote cleavage and activate several caspases, including caspase-3, caspase-6, caspase-7, caspase-8, caspase-9, and caspase-10 [9]. Similar to tBID (produced by caspase-8), truncated BID generated by granzyme B (gBID) translocates to the mitochondrial membrane and promote the release of mitochondrial death factors, Bax or BAK [2]. Because granzyme B and CD95L activate the death of BID-BAX/BAK-signal lines and they provide an independent mechanism for inducing apoptosis of the target cells. Thus, the lack of CD95 cells or overexpressing c-FLIP remained susceptible to CTL-induced death [7]. It will be important to determine the disruption of distal step along the path of death-signal by CD95L and granzyme B (such as loss of Bax/BAK) in order to reduce the deaths caused by type II CTL target cells that require a link between the extrinsic and intrinsic path to undergo apoptosis. The genetic resistance to CTL-induced death can be an important mechanism using which the tumor cells avoid immune surveillance.

Death receptor-ligand interactions may serve important physiological functions in tumor surveillance [6]. NK cells play an important role in controlling the tumor metastasis [14]. Administration of neutralizing monoclonal antibodies against either Apo2L/TRAIL or FasL significantly increases the liver metastasis of several tumor cell lines. While the inhibition of perforin-mediated killing also inhibits NK-mediated cytotoxicity [13] whereas it is possible to achieve complete inhibition only with combinations of anti-TRAIL and anti-FasL antagonistic antibodies [14]. The endogenous interferon-γ plays an important role in encouraging Apo2L / TRAIL expression on NK cells and T-cells [8]. These findings suggest that Apo2L/TRAIL and FasL can lead to a natural suppression of tumors by NK cells. FasL expression on cells other than NK cells may also contribute to tumor suppression [15].

Conclusion:

The alkaloid fraction of leaf extract of Achyrantes aspera Linn increases NK cells during breast cancer. The alkaloid fraction of dose 30 mg/kg bw and 100 mg/kg bw expressed better activity than cyclophosphamide and recommended for further investigations to be conducted as a potential anticancer agent.

REFERENCES