Benalu teh

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Cytotoxicity Effects of Benalu Tea Extract *(Scurrula oortina)* on Hella cells

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Abstract

Tea garden waste has been investigated in the form of parasite tea Squrula oortina Species Family: Loranthaceae from the Dempo Pagar Alam tea plantation, Lahat Regency, South Sumatra. Stem and leaf samples are then dried and powdered, then isolated with 70% water-ethanol subfraction. The extract was then dried by vacuum distillation at 70°C until a concentrated extract without ethanol was obtained. The results of the study were tea parasite extract, and the extract was tested for phytochemicals (monounsaturated fatty acids and double, theobromine derivates, quercetin derivates and Epicatekin derivates). Next, it would be taken to the Laboratory of Biological Sciences at Gajah Mada University to be tested for cytotoxic effects on Hella cells, with control positive doxorubicin. The results of the research were tea leaves extract IC50 = 2538 ug/mL and the standard of doxorubicin IC50 = 0.88 ug/mL so that it could be concluded that the parasite of the tea parasite had cytotoxic and proliferative effects.

Keywords. benalu tea, cytotoxic, Hella cells, proliferative

Introduction

Tea parasites grow and are found in unmanaged tea plantations, growing like parasites on trees (waste) because they are caused by bird droppings stuck to the tree. As a parasitic plant, parasites live by taking necessary nutrients owned by the host. Therefore, some of the compounds contained in the parasite resemble the host. Benalu, parasites which were initially thought to be waste are expected to have potential as chemo-preventive agents.[1,2]

The 2002 global cancer survey in Indonesia also showed that the incidence of lung cancer reached 28 per 100,000 population, 26 breast cancer per 100,000 people, colorectum cancer 23 per 100,000 people, cervical cancer 16 per 100,000 people and liver cancer 13 per 100 thousand people. Cancer is uncontrolled cell growth followed by the process of invasion into the

surrounding tissue and its spread (metastasis) to other parts of the body. The primary nature of cancer cells is characterized by loss of control of growth and development of cancer cells.[3]

The ideal anticancer drug should quickly kill cancer cells without harming healthy tissue. However, until now, there has not been found drugs with such criteria. In addition to the relatively large side effects, the price of these medicines is also high so that it is difficult to reach by most people in Indonesia. Efforts to treat cancer with traditional medicine are increasingly being carried out for reasons of lower costs, more accessible access, relatively small side effects, and can be mixed alone.[4,5]

Benalu, the parasite which was initially considered useless, turned out to be a parasitic tea *(Scurrulla oortina)* containing quercetin flavonoid compounds which have antitumor properties. The mechanism of the compounds in the parasite is likely through antioxidant activity. Quercetin can inhibit the expression of mutant p53 protein, tyrosine kinase, *heat shock protein* and *cyclooxygenase*, and show the same affinity with tamoxifen on estrogen receptors.[6]

Quercetin includes flavonoids useful as antioxidants, antimicrobial, antibacterial, antivirals, anti-inflammatory, anti-allergic, anti-mutagenic, anti-clastogenic, anticancer, anti-platelet, and others. On the other hand, it is also reported that flavonoids can cause DNA damage, mutations and apoptosis. The study of flavonoids is not only because of biological role, but because of its potential as a drug. [7]

Seeing the above it is necessary to do research on the parasitic waste of tea from the tea garden in Pagaralam after extracting it with polar (ethanol) solvent in testing the effects of inhibition of Hella cell growth.

Methods

The research design was an experimental study, namely by making a parasitic extract of tea, tested the effect of its cytotoxic on Hella cells and the effect of reducing LDL cholesterol in mice. Cytotoxic effects were read with Elisa reader, and cholesterol-lowering effects were carried out in white rats. ANOVA statistics distinguishes analysis of results.

The material used in this study was parasites of tea, which were obtained from the farmers' farms in the tea garden area in Dempo Pagar Alam, South Sumatra. In vitro test material are Hella

cervical human cancer cells (human epitheloid cervical carcinoma) and male white rat (Rattus novergicus).

A total of 2 kg of dried parasite simplicia of dried tea with 5% water content was macerated with 70% ethanol solvent. Each solvent was repeated three times 0.5 litres so as not to give spots on the test by thin-layer chromatography. Each macerated filtrate was collected and evaporated with a vacuum distillation vaporizer (70°C) until a thick extract was obtained, then continued drying with a vacuum pump in a desiccator until the weight remained. This is the result of research in the form of tea parasite extract.

Anti-proliferation and apoptosis tests were carried out by preparing three sterile plates, LAF was added with the following procedure; some of the wells in the plates were filled with 100 μL of Hella cell culture containing 2 x 104 cells with 1, 2 and 3 plate arrangements (in the appendix) incubated in a CO_2 incubator for 24 hours. Plate 1 is filled with control cells (cell + medium), treatment (cell + medium + extract 4000 to 7.8125 ug/mL), DMSO 1 to 6, doxorubicin (cell + medium + doxorubicin 50 to 1.5625 ug/mL. Plate 2 is filled with cell control (cell + medium), extract treatment (cell + medium + extract), extract control (cell + medium + extract), medium control only. Plate 3 is filled with cell control (cell + medium), DMSO 1 to 6 (cell + medium + DMSO), DMSO control (medium + DMSO), doxorubicin (cell + medium + doxorubicin), doxorubicin control (medium + doxorubicin), control medium (medium only). The three plates were put on a CO_2 incubator for 24 hours. Anti-proliferation and cytotoxic activity tests were carried out on Hella cancer cell culture using the trypan blue test method. Doxorubicin is used as a positive control and DMSO solution as a negative control. The number of live cells per well was calculated using the Dynatech MR5000 ELISA reader at a wavelength of 550 nm. IC50 values were determined from a graph of live cell per cent vs log concentration of test samples with Anova.

Results

Table 1. Extracts with a concentration of 7,812 - 4000 ug/ml

No	Treatment of extracts (ug/ml)	Per cent inhibition
1	4000	67,44
2	3000	56,41

3	2000	46,73
4	1500	33,17
5	1000	32,23
6	500	24,14
7	250	22,06
8	125	17,56
9	62,5	15,24
10	31,25	17,2
11	15,625	13,48
12	7,8125	17,08

The IC50 value is located between the extract concentrations of 2000ug / ml - 3000 ug/ml, i.e. at an extract concentration of 2538.86 ug/ml.

Table 2. Per cent inhibition of Doxorubicin and DMSO

No	Treatment of extracts (ug/ml)	Per cent inhibition
1	DMSO 1	16,22
2	DMSO 2	15,86
3	DMSO 3	13,27
4	DMSO 4	16,09
5	DMSO 5	15,82
6	DMSO 6	13
7	Doxo 50ug/ml	84,5
8	Doxo 25ug/ml	81
9	Doxo 12,5 ug/ml	57,77
10	Doxo 6,25 ug/ml	53,84
11	Doxo 3,125 ug/ml	50,68

The IC50 doxorubicin value is 0.88ug/ml. The strength of the cytotoxic between tea leaf parasitic extract is 1/2888 times doxorubicin.

Discussion

From the research results obtained IC_{50} for ethanol extract of tea, parasites were 2538 ug/ml, and IC_{50} values for positive control of doxorubicin were 0.88 ug/ml. The strength of the cytotoxicity between the tea leaf parasite extract is 1/2884 times doxorubicin.

Microscopic results of ethanol extract of parasitic tea leave 3000 ug/ml (slightly above the IC_{50} value) showed a Hella cell growth of about 50%, this is characterized by the appearance of cells that have fibres around them and thickened cell walls. Likewise, a little picture of a sample given doxorubicin 3,125 ug/ml showed a Hella cell whose cell wall was thickened. Microscopic picture of an enlarged preparation of 200x with an IC_{50} value measured by the Elisa reader model 680XR micro-plate reader S/N 000. [8-10]

The toxicity test of bitter melon and bitter leaf extract was carried out by direct counting method with the help of trypan blue staining into 96 wells micro-plate, which contained 100 ul of test cells with 2x 10^4 density, added 100 UL of test extracts at various concentration ratings (1000, 500, 250, 100, 50, and 10 µg/ml) by triplicate. As a control used culture media that are considered to have 100% growth. Culture containing the test material was then incubated for 24 hours. At the end of the incubation of each wells counted the number of living cells using *trypan blue* (which is blue means the cell has died). Levels that can inhibit growth of up to 50% (IC_{50}) are determined by probit analysis. IC_{50} values were further analyzed by ANOVA. The research results of the researchers showed the IC_{50} value of *M.Charantia.L* ethanol extract and *Phylantus niruri* in Hella cells respectively 51.56 ug / ml; 87.73 ug/ml. [11-14]

With IC_{50} of such a large tea parasite extract of 2538 ug/ml and doxorubicin of 0.88 ug/ml, there is a big difference and this is not apoptosis (cell death) but the tea parasite extract has a proliferation effect on Hella cancer cell growth.[15-17]

Conclusion

Tea parasite extract has potential as an anti-proliferative.

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