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Potential Analysis of Cottonwood Parasite (*Dendropthoe pentandra*) Stem Extract in Decreasing of Mutant P53 Protein Expression on Cervical Cancer Cell (HeLa Cells) in Vitro

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ABSTRACT

Cervical cancer is the second most common type of cancer in women and the most common cause of mortality related cancer in developing countries.In 2005, cervical cancer leads to over 250,000 deaths in the world.Treatment in cancer are including surgery, chemotherapy and radiotherapy. Surgery can not be done for the metastasized cancer, while chemotherapy and radiotherapy treatment can cause various side effects.Cottonwood Parasite (*Dendrophthoe pentandra*) stem contains quercetin 39.8 mg/g. Quercetin acts as an anticancer on cell cycle regulation, interacts with estrogen receptor type II, inhibits tyrosine kinase enzymes, and suppresses the expression of mutant p53 protein.This research aims to know the effect of Cottonwood Parasite stem extract against mutant p53 expression on HeLa cell (cervical cancer).Cottonwood parasite stem is obtained from extraction and followed by evaporation with ethanol 70%.HeLa cells were divided into 4 groups:HeLa cells without treatment(A), HeLa cells treated with 25µg/ml extract concentration(B), 50µg/ml(C), 100µg/ml(D). Immunocytochemistry method was performed using monoclonal antibody to mutant p53 to measure the expression of mutant p53 level as an indicator of apoptosis on HeLa cells, by examining the appearance of brown colour under light microscope on 1000x magnification.This results showed that cottonwoods Parasite stem extract was decrease mutant p53 protein expression in HeLa cells culture.Based on these facts, quersetin which are found mainly in the cottonwood parasite is likely to be developed as an anticancer drug that is promising in the future, either as agent chemoprevention or co-chemotherapy (companion agent of chemotherapy).

Keywords: Cottonwood parasite, cervical cancer, p53 mutant, quersetin

INTRODUCTION

Cervical cancer is the second most common type of cancer in women and the most common cause of mortality related to cancer in developing countries. It attacks 16 of 100,000 women per year and kills about 9 out of 100,000 women per year.[1] In 2005, cervical cancer leads over 250,000 deaths in the world and without adequate managements, it expected that the death rate will increase as much as 25% in the next 10 years.[2]

Conventional treatments in cancer are including surgery, chemotherapy, and radiotherapy. Therapeutic cancer surgery can not be done for the metastasized cancer, while chemotherapy and radiation treatment can cause various side effects.[3]

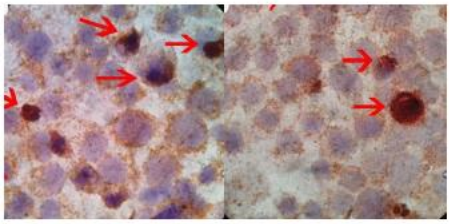
Cottonwood parasite (*Dendrophthoe pentandra*) stem contains quercetin 39.8 mg/g. Quercetin acts as an anticancer on cell cycle regulation, interacts with estrogen receptor (ER) type II, inhibits tyrosine kinase enzymes, and suppresses the expression of mutant p53 protein.[4]

METHOD

This study uses cervical cancer cell line (HeLa cells) which is cultured in the Laboratory of Biomedical, Faculty of Medicine, University of Brawijaya. The extract from stem of cottonwood parasite is obtained from extraction and followed by evaporation with ethanol 70%.HeLa cells were divided into 4 groups:Negative

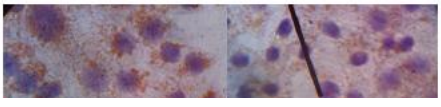
control group or HeLa cells without treatment, HeLa cells treated with 25 mg/ml extract concentration, HeLa cells treated with 50 mg/ml extract concentration, HeLa cells treated with 100 mg/ml extract concentration.

Once given the treatment, cell cultures were incubated for 24 hours. Then, cell cultures were fixed and then performedstaining.Immunocytochemistry method was performed using monoclonal antibody to mutant p53 to measure the expression of p53 level as an indicator of apoptosis on HeLa cells, by examining the appearance of brown colour under light microscope on 1000x magnification.Immunocytochemistry staining is observed as brown spots in nuclear cell.



A

B



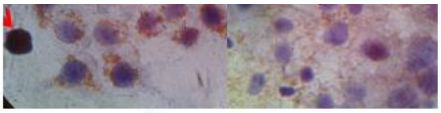
RESULTS

The calculation was done by counting the number of HeLa cells that express mutant p53 protein in each field of view. Each treatment was calculated as many as 20 field of view with 1000x magnification. The appearance of HeLa cells with mutant p53 protein will be seen asbrown color on the nucleus. Whereas the normal HeLa cell will be blue-purple colour on the nucleus. The results of the calculation can be seen in the following table:

Tablel 1.Results of Immunocytochemistry

Treatment	Average (per 20 field of view)
Negative Control	86
25 mg/ml	67
50 mg/ml	58
100 mg/ml	47

From the table above, it can be seen the effect of cottonwood parasite stem extracton the number of HeLa cells showing the expression of mutant p53 protein. The effect can be seen in the difference of mutant p53 protein expression between the control group and



C
D

Figure 1. Immunocytochemistry staining was performed on mutant p53 protein. Mutant p53 protein expressions were decrease after incubated with parasite cottonwoods. Mutant p53 protein expression on normal HeLa cell or control (A); 25mg/ml parasite cottonwoods (B); 50mg/ml parasite cottonwoods (C); 100mg/ml parasite cottonwoods (D).

the treatment group. The decrease of mutant p53 protein expression in the treated group increasee perpendicularly with doses concentration from the extract.

Data Analysis

In calculating the results of this study used 95% confidence interval (α = 0.05). Statistical data analysis using One-Way ANOVA because in this studythe data used is ratio, which has one dependent variable and one independent variable with some groups with a single differentiating factor (concentration ofcottonwood parasite stem extract). Subsequent analysis was done by Pearson correlation test to analyze the relationship between the extract of parasite cottonwood on the expression of mutant p53 protein in HeLa cell. After that, linear regression analysis

was done to analyze percentage of the effect of parasite cottonwoods extracts on the decline of mutant p53 protein expression

Analysis of One-Way Anova

Data analysis was performed with One-way Anova test using SPSS16.0 program for Windows. The analysis aims to determine the significantdifference in parasite cottonwood stem extractbetween groups and to know concentration of parasite cottonwood stem extract which provide a significant difference in the decrease of expression of mutant p53 protein. Before One-way Anova test, data requirements test consists of normality test and homogeneity tests was carried out. Both of the tests must have a probability p value > 0.05 to be able to proceed in One-way Anova test. Shapiro-Wilktest was used in normality test with result is significant probability (p value > 0.05). This results indicate that the distribution of data is normal. Homogeneity test result probability p value > 0.05 which means that the variance of the data is normal. Because all of test results have significant value, it can proceed to one way Anova test. In this test, the p value = 0.000 (p <0.05), so it can be concluded that "at least

Comparison between treatment		Signifi-cance value	Conclusion
control	Doses 25	0,084	-
	doses 50	0,017	Signifance
	doses 100	0,003	Signifance
doses 25	Control	0,084	-
	doses 50	0,337	-
	doses 100	0,051	Signifance
doses 50	Control	0,017	Signifance
	doses 25	0,337	-
	doses 100	0,240	-
doses 100	Control	0,003	Signifance
	doses 25	0,051	Signifance
	doses 50	0,240	-

2. The insignificant difference between the doses of 25 mg/ml compared with a doses of 50 mg/ml. But there is a difference that approached significance (p=0.051) when compared with the doses of 100 mg/ml.
3. There is no significant different between doses of 50 mg/ml with doses of 100 mg/ml.

Pearson Correlation Test

To assess relationship between the

there are significantly differences in the expression of mutant p53 protein in HeLa cell between the two groups." Furthermore, *Post Hoc Multiple Comparison* (Tukey HSD) analysis was performed to determine the comparative differences between the treatment group and which groups have significant differences.

Table 1. Post Hoc Multiple Comparison Analysis

In the table above, differences value is significant if p value <0.05.From the analysis, it can be concluded that :

1. There is no significant different between control andtreatment doses of 25 mg/ml. The new difference value is significant at doses 50 and 100 mg/ml.

extract of parasite cottonwood with mutant p53 protein expression in HeLa cell was performed Pearson correlation test. From these test, it is known that the Pearson correlation test have significance value 0.001 (p <0.01), which means there is a significant relationship between parasite cottonwoods stem extract and the expression of mutant p53 protein. Value of Pearson correlation is -0.808. The correlation is negative, which means increasing dose ofparasite cottonwood stem extract will cause further decline in the expression of mutant p53 protein (negative correlation).

Linear Regression Test

Linear regression analysis was performed to find out the magnitude effect of parasitecottonwood stem extract on the

decrease of mutant p53 protein expression,. The results of the analysis was read in R square. These results are multiplied by 100% to turn it into a percentage. final results of the analysis is 69.4% which means parasite cottonwood stem extracts can give effect to the decrease of mutant p53 protein expression as much as 69.4%.

DISCUSSION

One of the causes of malignancy is the failure or inactivation of tumor supressor gene p53. Tumor supressor gene p53 is a recessive gene on short arm of chromosome 17 acting on the p53 wild-type allele and function to inhibits growth and differentiation of cells thus preventing the onset of cell transformation that leads to malignancy. If there is damage or mutation of the tumor supressor genep53 by genetic and environmental factors, unstable mutant p53 protein is formed and unable to inhibit growth from the G1 phase to S phase so that if there is any damage in the cells the damage can not be repaired. As the result, the damage cells continue to differentiate and initiate the process of malignancy in epithelial cells. These proliferation continuously. According to Lamson, et al., quercetin, in certain concentrations, can suppress the expression of mutant p53 proteins are formed by breast cancer cells until undetectable in these cells.[4] The mechanism is through inhibition at the gene that encodes mutant p53 protein. If this gene is inhibited, the production of mutant p53 protein will decline. Inhibition of the p53 protein expression caused the cells suspended in the G2-M phase of the cell cycle. In this phase.there is examination of DNA damage (G2-M DNA damage checkpoint). This checkpoint make sure the cells not to initiate mitosis until DNA damage is repaired. Cells unable to repair after entering G2-M phase checkpoint will undergo apoptosis.[5]

Previous studies was conducted to determine the effects of quercetin in downregulating mutant p53 protein expression in breast cancer cell line. We repeatedthis study, but using a different cell-line cancers, called HeLa cells (cervical cancer). The results of the experiment can be seen in Table 1. From this table, it can be seen that, in average, the cell generally show a significant

mutations tend to occur in the gene at codon 132 to 281. Mutations in the p53 protein is the most frequent genetic abnormalities occur in human cancer. In a study assessing correlation between mutant p53 protein expression with prognosis of cervical cancer, there was found the mutant p53 protein expression percentage of 47.8% on the total 399 cases.[4]

Parasite cottonwoods stem extract has a major content of flavonoid, that is quercetin. Parasite cottonwood has the highets composition of quercetin than other plant, i.e 39.8 mg/g. Quercetin was believed to have an important role in lowering the expression of mutant p53 protein. Physiologically, P53 protein is a pro-apoptotic proteins. However, more than 50% of cancers have mutations of this protein. These mutations make proteins unable to work properly and result in cell reduction of cells that express mutant p53 protein in the extract of parasite cottonwood stem. Any increase in doses of parasite cottonwood stem extract will decrease the expression of mutant p53 protein in HeLa cell.[6]

However, there are some results that showed no change between the control andtreatment, such as between the control repetition 1 with a treatment of 25 mg/ml repetition 1. *Post Hoc Statistical Tests Multiple Comparison* (Tukey HSD) results there was no significant difference in the comparison control group with a treatment of 25 mg/ml. This not significant results also repeated in the comparison between the doses of 25 mg/ml to 50mg/ml doses, a doses of 50 mg/ml with a doses of 100 mg/ml. In average the cells number is decrease, but the statistical tests provide results which are less significant. The new

dose was significant in comparison with the control doses of 50 mg/ml and 100 mg/ml. In comparison between doses of 25 mg/ml with doses of 100 mg/ml, statistical tests showed the value approached significance ($p=0.051$, significant if $p<0.05$). From these results, it can be concluded that the parasite cottonwood stem extract needs certain increase doses to be able to give meaningful effect in suppressing expression of mutant p53 proteins. Comparison of doses approached significance was between the doses of 25 mg/ml with a doses of 100 mg/ml, with increasing doses of 75mg/ml. Significant results come from a comparison of control with a doses of 100 mg/ml, with increasing doses of 100 mg/ml. From this data, it can be concluded that the doses of parasite cottonwoods stem extract will give significant effect on decline of expression of mutant p53 proteins at dose > 75 mg / ml.

The results of this study are almost similar to previous studies on breast cancer cells (MDA-MB 468). In this study, the suppression effect to mutant p53 protein was reach at a doses of 30 mg/ml.

CONCLUSION

This results showed that parasite cottonwoods stem extract were suppress mutant p53 protein expression in HeLa cells culture.Based on these facts, quersetin which are found mainly in the cottonwood parasite is likely to be developed as an anticancer drug, either as agent chemoprevention or co-chemotherapy (companion agent of chemotherapy). Moreover, it can increase the sensitivity of cancer cells to chemotherapy as well as reduce resistance and side effects. The development and cultivation of cottonwood parasites as well as further processing into product has properties necessary for optimal chemotherapeutic agents and also promising modality in the future.

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Increased concentrations of quercetin provides a dramatic effect, and cause the expression of mutant p53 protein to almost undetectable levels at doses of 75 mg / ml.

In the linear regression test, the analysis value is 0,694 or 69.4%. That's mean, the effect of parasite cottonwood stem extract to the decrease of mutant p53 protein expression in HeLa cells amounted to 69.4%. 20.6% are caused by other factors, such as temperature, humidity, durability of the cell, etc. However, 69.4% can be quite significant since parasite cottonwood stem extracts give effect more than 50%. However, further research is needed to assess the effectiveness of parasite cottonwood stem extracts and to determine an effective doses in humans.

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Jurnal Ilmiah Mahasiswa Kedokteran Indonesia

Pengaruh Induksi *Cathepsin K* terhadap Pembentukan Imunoglobulin (IgG) Anti-*Cathepsin K*, Osteosit, dan Kadar *Alkaline Phosphatase* (ALP) pada Tikus Putih (*Rattus norvegicus* L.) Betina Galur Wistar Pascaovariektomi

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ABSTRAK

Osteoporosis adalah suatu *"silent disease"* yang dapat melemahkan tulang dan menyebabkan fraktur. Dua dari lima penduduk Indonesia berisiko terkena osteoporosis dan diperkirakan pada tahun 2025 angka tersebut meningkat tiga kali lipat. Saat ini telah ditemukan obat-obatan yang berfungsi sebagai inhibitor *cathepsinK* yang menunjukkan potensi besar dalam menurunkan tingkat osteoporosis.*Cathepsin K* berperan penting dalam destruksi jaringan, *remodelling*, dan perusakan kartilago tulang.Penelitian ini ditujukan untuk membuktikan pengaruh pemberian kandidat vaksin berbahan dasar *cathepsin K* terhadap penurunan kecepatan resorbsi tulang pada tikus Wistar (*Rattus norvegicus*) yang diovariektomi. Tikus putih Wistar betina berusia 10-12 minggu dikelompokkan menjadi 5 kelompok:kontrol (-), kontrol (+) yang diovariektomi, kelompok perlakuan yang diovariektomi dan diberikan *cathepsin K* 50ng/200 µL, 100 ng /200 µL, dan 200 ng/200 µL. Pembedahan dilakukan pada hari ke-30 dan dilakukan pengukuran titer IgG anti-*cathepsin K*, penghitungan jumlah osteosit, dan pengukuran kadar ALP serum. Uji ANOVA menunjukkan bahwa pemberian kandidat vaksin*cathepsin K* yang ditambahkan dengan CFA-IFA secara bermakna meningkatkan titer IgG anti-*cathepsin K* dalam serum (p=0,00).Pemberian *cathepsin K* dosis 50ng/200 µL, 100 ng /200 µL, dan 200 ng/200 µL tidak mengurangi jumlah osteosit secara bermakna. Pemberian *cathepsin K* dosis 50ng/200 µL, 100 ng /200 µL, dan 200 ng/200