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The Effect of Curcumin Administration on p53 and Caspase-3 Expression in Cervical Cancer HeLa Cell Culture

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ABSTRACT

Introduction: Cervical cancer is a type of cancer with 605,000 new cases globally each year, with a mortality rate of 340,000. Curcuma longa, or curcumin, has the potential to enhance apoptosis in cervical cancer cells through the NF-kB-p53-caspase-3 pathway.

Material and Methods: This in vitro experimental study employed a Completely Randomized Design (CRD) using HeLa cell cultures derived from cervical adenocarcinoma (ATCC® CCL2™). Cells were grown in Dulbecco's Modified Eagle Medium (DMEM) and treated with curcumin at concentrations of 25 µg/mL, 50 µg/mL, 100 µg/mL, 150 µg/mL, and 250 µg/mL. Cytotoxicity was assessed using the CCK-8 assay, apoptosis via flow cytometry, and p53 and caspase-3 expression using immunofluorescence. Statistical analysis was performed with IBM Statistics SPSS 25.

Results: There was an increase in the percentage of apoptosis, p53 expression, and caspase-3 expression. At a dose of 100 µg/mL, total apoptosis increased significantly, reaching nearly 25% (p=0.000). P53 expression significantly increased at curcumin doses of 50 µg/mL and 100 µg/mL (p=0.021). There was a significant increase in caspase-3 expression, reaching approximately 86.2%, with a maximum effect at a dose of 100 µg/mL (p=0.002).

Conclusion: Curcumin shows promising potential as an anticancer agent by reducing cell viability and enhancing apoptotic activity in HeLa cervical cancer cells.

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INTRODUCTION

Cancer is the second leading cause of death in the world after cardiovascular disease. Based on data from the World Health Organization (WHO) and GLOBOCAN, there were around 19 million new cases and 9 million deaths from cancer worldwide in 2020. In Indonesia, cervical cancer is still a serious health problem in women where high incidence and mortality rates are found with 100 new cases and 57 deaths every day, cervical cancer cases in Indonesia are ranked second in terms of the total number of sufferers and the number of deaths after breast cancer [1–4].

The immune system and microbiota of the human urogenital tract play a role in maintaining the health of the urogenital system. Disruption of the microbiota balance can cause urogenital tract infections, which can lead to cervical cancer. Human papillomavirus (HPV) infection is considered the most important risk factor associated with the development of cervical cancer, and there are 99.7% of invasive cervical tumors worldwide consisting of all squamous cell cancers and adenocarcinomas [5–10].

HPV virus has 2 oncogene proteins, namely E6 and E7, which play a role in the imbalance of apoptosis and cell cycle. The E6 protein plays a role in the inactivation of the p53 tumor suppressor protein by inducing degradation of the protein, with a decrease in p53 protein levels providing effects on other proteins that can affect the process of cell proliferation and apoptosis [11–13].

Curcumin has the ability to increase apoptosis of cervical cancer cells through the NF-kB-p53-caspase-3 pathway. First, curcumin inhibits NF-kB activation resulting in the suppression of genes that play a role in tumorigenesis such as TNF, COX-2, cyclin D1, c-myc,

MMP-9, and interleukin. Second, curcumin plays a role in controlling the cell cycle and stimulating apoptosis through the regulation of p53 and caspase 3. Third, curcumin is an autophagy modulator and has an inhibitory effect on tumor angiogenesis and inhibits metastasis through suppression of various growth factors including VEGF, COX-2, MMPs, and ICAMs [14,15].

The results of Kurniawan C's study (2013) reported that curcumin at a dose of 184.5 µg/ml caused cell death of half the number of cells it induced. The study also found that some cell death was in the form of apoptosis. Meanwhile, Mirani et al. (2011) on the cytotoxic effects of turmeric rhizomes stated that the dose of turmeric rhizome extract that gave the greatest cytotoxic effect was at 250 µg/ml, which was able to kill 74.72% of HeLa cells, while the lowest was at 25 µg/ml, which killed 44.72% of HeLa cells. Based on the description above, researchers are encouraged to conduct further research on the activity of curcumin compounds as antiproliferative and apoptosis inducers in cervical cancer (HeLa cell lines). In this case, researchers want to explore the effect of curcumin administration on p53 and caspase-3 in cervical cancer cells (HeLa cell lines) in vitro.

MATERIAL AND METHODS

This research is a true laboratory experimental study conducted in vitro with the assumption that all populations are the same and have homogeneous criteria and the same characteristics. This study used a research design in the form of a Completely Randomized Design (CRD). A completely randomized design is a type of experimental design where treatments are given randomly to all experimental units, employing a Completely Randomized Design (CRD).

The samples used in this study were HeLa cell cultures grown in Dulbecco's Modified Eagle Medium (DMEM). The HeLa cells were obtained from a patient with adenocarcinoma cervical cancer (ATCC® CCL2™).

The number of cells used in this study was 10,000 cells/100 µl/well for the CCK-8 assay (cytotoxicity), 1,000,000 cells/1,000 µl/well for flowcytometry analysis (apoptosis), and 20,000 cells/150 µl/well for immunofluorescence (expression of p53 and caspase-3). The doses of curcumin administered to the HeLa cells were 25 µg/mL, 50 µg/mL, 100 µg/mL, 150 µg/mL, and 250 µg/mL. Data on cytotoxicity, apoptosis, p53 expression, and caspase-3 expression were analyzed using IBM Statistics SPSS 25 software.

RESULTS

There was an increase in the percentage of apoptosis, p53 expression, and caspase-3 expression. At a dose of 100 µg/mL, total apoptosis increased significantly, reaching nearly 25% (p=0.000) (Fig.1 and Fig.2). p53 expression significantly increased at curcumin doses of 50 µg/mL and 100 µg/mL (p=0.021) (Fig.3). There was a significant increase in caspase-3 expression, reaching approximately 86.2%, with a maximum effect at a dose of 100 µg/mL (p=0.002) (Fig.4).

DISCUSSION

Cervical cancer is initiated by infection with HPV oncoproteins, especially E6 and E7 which play a major role in altering host cellular function. HPV overexpresses E6 and E7 oncoproteins to disrupt the normal function of tumor suppressor genes in the host. After integration, the viral proteins begin to damage host cells. Host cells have developed specific mechanisms to repair damage to their DNA through the DNA damage response. Under certain conditions, if cells are unable to repair DNA damage, apoptosis will occur. However, in HPV-associated cancers, the viral proteins E6/E7 disrupt cell cycle checkpoint control by inhibiting cyclin-dependent kinase (CDK) inhibitors (p21, p27, p16) and destroying p53 and retinoblastoma (pRb). Degradation of p53 by the E6 oncoprotein induces apoptosis allowing cells to continue replicating.

HeLa cell viability was measured at 24 hours. HeLa cells were given various doses of curcumin, namely 0µg/mL (control), 25µg/mL, 50µg/mL, 100µg/mL, 150µg/mL and 250µg/mL. HeLa cell viability was measured using the CCK-8 assay. After 24 hours of treatment, curcumin showed anti-proliferative activity as indicated by a decrease in the number of viable cells compared to the 0µg/mL control group. A significant difference was found at a dose of 0µg/mL compared to a dose of 100µg/mL ($p<0.05$, ANOVA test and Post Hoc LSD test) [16–18].

These results are in line with previous studies that show that curcumin can reduce cell growth, induce apoptosis, and stop the cell cycle in cervical cancer cell lines. Nanoparticle treatment modulates entities such as miRNA, transcription factors, and proteins related to carcinogenesis.

The results of Kurniawan's study (2013) reported that curcumin at a dose of 184.5 µg/ml caused cell death of half the number of cells it induced. The study also found that some cell death was in the form of apoptosis. Meanwhile, Mirani et al. (2011) on the cytotoxic effects of turmeric rhizomes stated that the dose of turmeric rhizome extract that gave the greatest cytotoxic effect

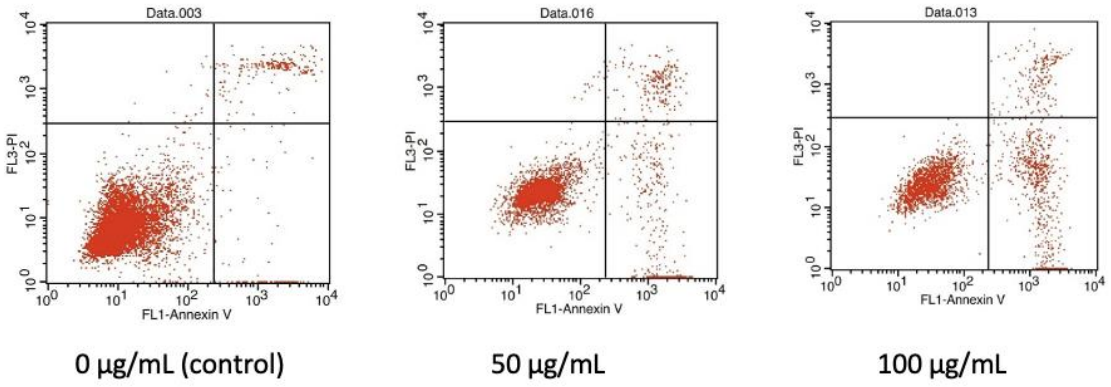


Figure 1. Apoptosis Test Results from Flowcytometry Examination on HeLa Cells

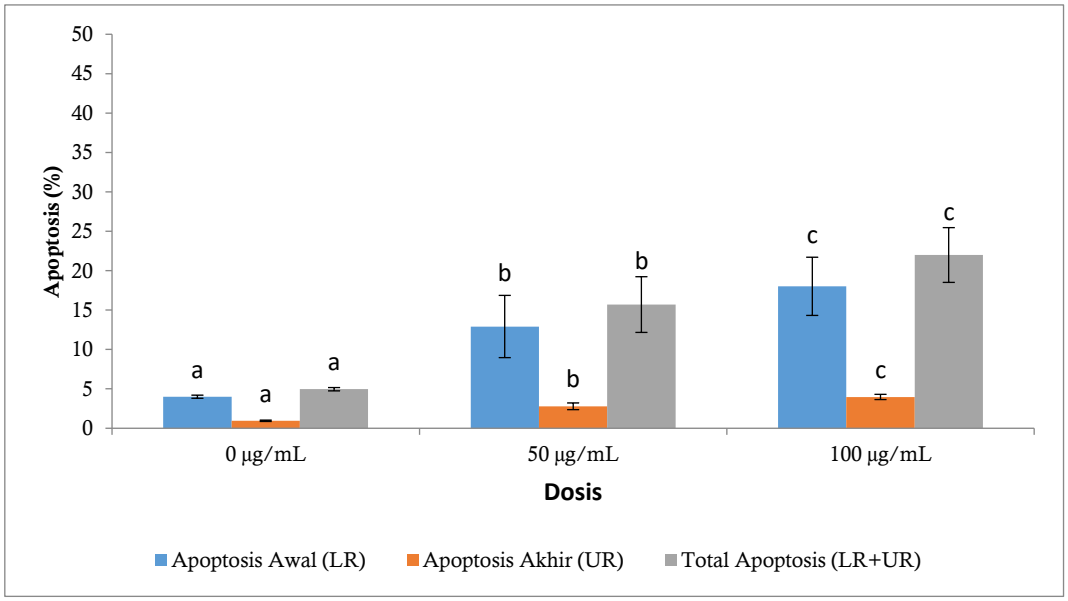


Figure 2. Analysis of Absorbance Value Based on Curcumin Dose

was at 250 µg/ml, which was able to kill 74.72% of HeLa cells, while the lowest was at 25 µg/ml, which killed 44.72% of HeLa cells.

Curcumin significantly increased total apoptosis in HeLa (LR) and (UR) cells ($p<0.05$, ANOVA test). Along with the increase in dose, total apoptosis in HeLa cells also increased significantly, especially at a dose of 100µg/mL compared to the 0µg/mL group ($p<0.05$, Post Hoc LSD test) [19,20].

To determine the apoptosis activity, the expression of p53 as an indicator of apoptosis was observed. HeLa cells were given curcumin at three different doses for 24 hours, namely control (0µg/mL) and IC50 (50µg/mL, 100µg/mL). All three doses showed an increase in apoptosis activity as indicated by an increase in p53 expression as pro-apoptosis ($p<0.05$, One-Way ANOVA). Based on these data, it was found that along with the increase in the dose of curcumin, p53 expression also increased. The most significant increase in p53 expression was at a dose of 100µg/mL when compared to the 0µg/mL group (p-value <0.05 post Hoc LSD) [21].

The results of this study with p53 observation through immunofluorescence staining, showed an increase in p53 expression followed by the administration of an increasing dose of curcumin. This increase in p53 expression indicates an increase in apoptosis induction in Hela cells. The results of other studies show that cell viability testing after treatment with curcumin shows that cytotoxic activity is selectively higher in cervical cancer cells containing p53.

HeLa cell apoptosis was identified by administering three doses of curcumin for 24 hours. The doses used were control (0µg/ml) and IC50 (50µg/mL, 100µg/mL).

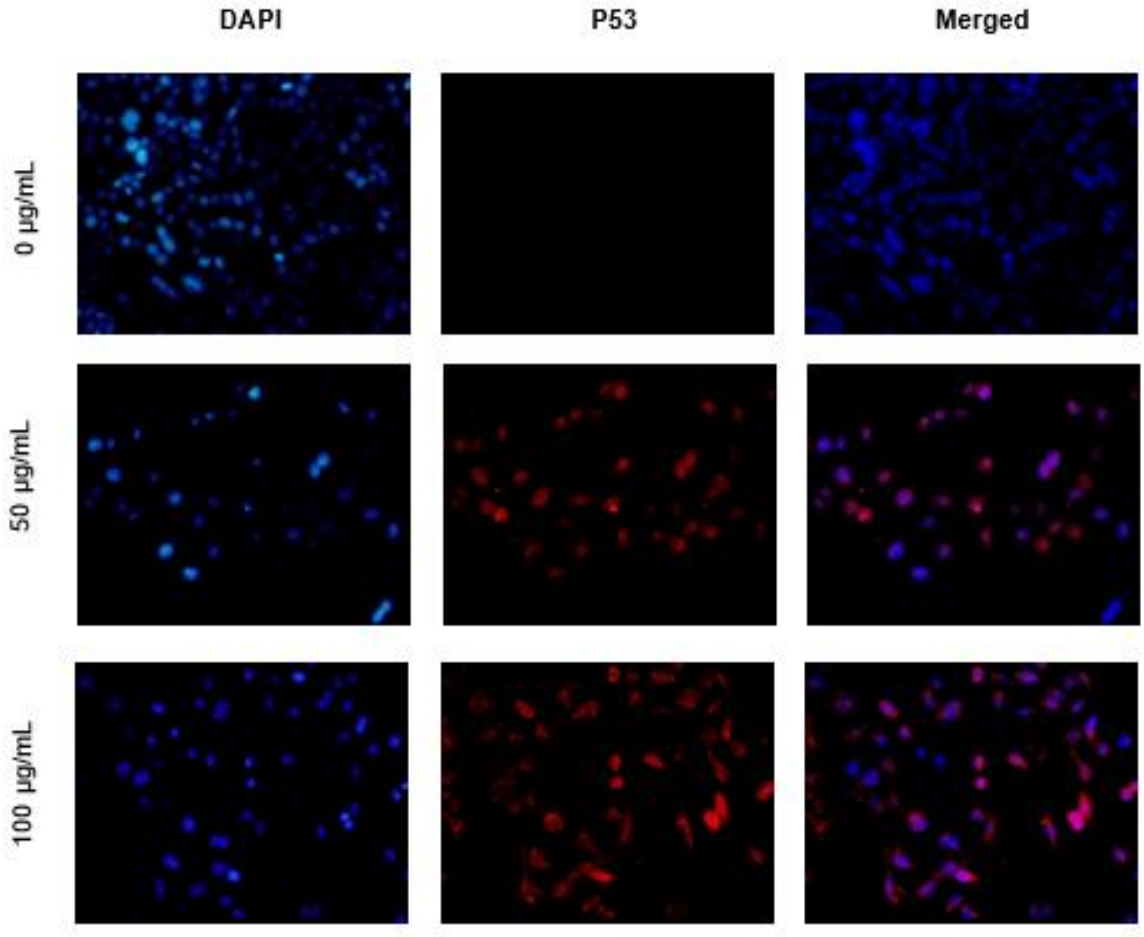


Figure 3. **p53 Expression on Immunofluorescence Examination**

Caspase-3 expression was measured using the immunofluorescence method with rabbit antibodies. Administration of curcumin at doses of 50 µg/mL, and 100 µg/mL showed an increase in caspase-3 expression at 24 hours in the treatment group compared to the control group 0µg/mL ($p<0.05$) [22].

The results of this study are in line with previous studies. Research by Zhao et al 2023 showed that curcumin was demonstrated through transwell assays showing that treatment with curcumin inhibited the invasion and migration of HeLa and CaSki cells in a dose-dependent manner compared to control treatment. Curcumin also decreased the expression of Bcl-2, N-cadherin, and Vimentin and increased the expression of Bax, C-caspase-3, and E-cadherin in both cell lines. Further studies showed that curcumin also selectively inhibited the expression of viral oncoproteins E6 and E7.

CONCLUSION

Curcumin shows promising potential as an anticancer agent by reducing cell viability and enhancing apoptotic activity in HeLa cervical cancer cells. The antiproliferative activity of curcumin is characterized by decreased cell viability and the effect of increasing apoptosis activity in HeLa cells which are cervical cancer cells. Curcumin with various doses significantly increased the expression of pro-apoptotic proteins p53, caspase-3, total apoptosis, and decreased HeLa cell viability. The results of this study indicate that curcumin has promising potential as an anticancer that can be studied and observed further.

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CONFLICT OF INTEREST

The authors declare there is no conflict of interest.

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