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ARTIKEL PENELITIAN

Time- and Concentration-dependent Cytotoxicity of Piperine on Hela Cells

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Abstrak

Piperin merupakan alkaloid utama dari *Piper nigrum* dan *Piper longum* yang diduga memiliki aktivitas antikanker. Sifat antikanker piperine telah terbukti menargetkan jalur yang terlibat dalam siklus sel dan apoptosis dan telah dibuktikan pada berbagai jenis kanker, namun penelitian pada kanker serviks masih terbatas. Penelitian ini bertujuan melihat pengaruh konsentrasi dan durasi pemberian piperin terhadap viabilitas sel Hela. Penelitian ini dilaksanakan dengan menggunakan metode MTT. Konsentrasi piperin yang digunakan yaitu 1.000 ; 500; 250; 125; 62.5 µg/mL dengan lama pemberian 24 dan 48 jam. Dari penelitian ini didapatkan nilai IC_{50} 711.21 µg/mL dan 419.75 µg/mL setelah inkubasi 24 dan 48 jam. Dapat disimpulkan bahwa lamanya waktu pemberian dan konsentrasi piperin mempengaruhi viabilitas sel HeLa ($p<0.05$).

Kata kunci: Piperin, HeLa, Viabilitas sel

Abstract

Piperine, the primary alkaloid of *Piper nigrum* and *Piper longum*, is believed to have anticancer properties. Although research on cervical cancer is still in its early stages, piperine's anticancer capabilities have been established in numerous types of cancer and have been shown to target pathways involved in the cell cycle and apoptosis. This study aims to determine how piperine administration time and concentration affect the viability of Hela cells. The MTT method was used to conduct this study. The concentration of piperine utilized was 1,000; 500; 250; 125, and 62.5 µg/mL, and it was administered for 24 and 48 hours. The IC_{50} values, obtained after incubation for 24 and 48 hours, were 711.21 µg/mL and 419.75 µg/mL, respectively. We may conclude that the viability of HeLa cells is influenced by piperine in a time- and concentration-dependent manner ($p<0.05$).

Keywords: Piperine, HeLa, Cell Viability

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INTRODUCTION

In Southeast Asia, cervical cancer ranks second in terms of frequency among cancers in women and is the primary cause of cancer-related deaths in low- and middle-income nations. Cervical cancer is the leading cause of cancer death for women and the fourth most common disease in terms of diagnoses, according to Globocan 2018 (1). Cervical cancer is the second most common cause of cancer among Indonesian women between the ages of 15 and 44. According to estimates, 87.0% of women in the general population have developed invasive cervical cancer, and 4.0% of them had HPV infections (2).

Cervical intraepithelial lesions gradually develop from normal cervical epithelium, and hrHPV infection is the primary cause of cervical cancer. Changes in the host genome brought about by hrHPV infection of the cervical epithelium can inhibit tumor suppressor genes and increase oncogene activity (3). Three transformation proteins, namely E5, E6, and E7, are encoded by HrHPV(*high-risk HPV*). The E5 protein can prevent apoptosis and ensure that epithelial cells continue to replicate. But if it turns into cancer, its expression vanishes (4). It is believed that the HPV E5 protein has a role in the early phases of process of cervical cancer start (5). The E6 and E7 proteins are consistently expressed in intraepithelial lesions and after they progress to malignancy (6).

It is still difficult to produce natural substances as cancer medications, particularly when it comes to finding selective cancer medications. Since ancient times, people have used piperine, a piperidine alkaloid substance derived from *Piper nigrum* and *Piper longum*, as a popular spice (7). The chemopreventive effect of piperine inhibits breast stem cell proliferation and does not cause toxicity to differentiated cells (8). Piperine induces apoptosis and increases the percentage of cells in the G2/M phase in 4T1 cells (9).
There is currently a shortage of research on piperine's cytotoxic effects on cervical cancer cells. This work aims to determine how piperine concentration and time affect the viability of HeLa cells. The HeLa cell line is used in the present research to investigate cervical cancer cell viability. Cell viability was ascertained using the MTT technique, which is a nonradioactive colorimetric assay.

METHOD

Cell Culture

HeLa cell culture is the biological material that is utilized. Certified HeLa cells from the Biomedical Laboratory of the Andalas University Faculty of Medicine served as the study's sample source. The HeLa cells came from the same parent, were culturing-ready, and reached 80–90% confluence, with around 2×104 cells per well. HeLa cell-containing wells were split into treatment and control groups. HeLa cells were cultured at 37 ºC with 5% CO2 in DMEM media supplemented with 10% FBS, 100 IU/ml penicillin, and 10 µg/ml streptomycin.

Viability Assay

Cell viability was tested with 3-(4,5-dimeylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) to evaluate cell viability. HeLa cervical cancer cells were cultured in a 96-well plate at a density of $2x10^4$ cells/mL cells per well. The concentration of piperine(Sigma Aldrich) used is 62.5; 125; 250; 500; and 1.000 µg/mL for 24 hr and 48 hr administration of piperine. The cells were washed and treated with 25 µl of MTT reagent containing 5 mg/mL per well. The absorbance was measured at 595 nm using an ELISA reader (Bio-Rad) and was compared with the control cells. To determine cell viability, percent viability was calculated as [(absorbance of drug-treated) sample/(control absorbance)] × 100.

Statistical Analysis

Data are presented as the mean ± SEM. One-way ANOVA was used to assess statistical significance. A difference that was deemed statistically significant was defined as $p<0.05$.

RESULT AND DISCUSSION

Piperine, the most common dietary alkaloid, is found mainly in the Piperaceae family species *Piper nigrum* L. and *Piper longum* L. (10). Piperine is an alkaloid chemical with a variety of pharmacological actions, including antioxidant, anti-inflammatory, immunomodulatory, anticonvulsant, antimutagenic, antimycobacterial, antiamoebic, and anticancer (11). Piperine has been demonstrated to have anti-cancer effects in animal and cell cultures (12). Cell cytotoxicity and proliferation assays are mainly used to test the response of cells to a medication or chemical agent. To screen the outcome of a study, researchers employ many sorts of assays (13).

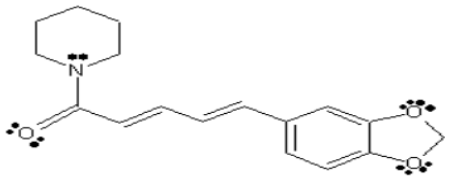

Figure 1. Chemical Structure of Piperine

A sample's number of healthy cells is defined as cell viability (14). The MTT assay was used to measure cell viability. MTT tests are typically performed by adding MTT solution to culture cells and incubating them for many hours. The MTT assay involves mitochondrial succinic dehydrogenases to convert the tetrazolium dye to formazan, although cytosolic enzymes such as nicotinamide adenine dinucleotide (NADH) reductase and flavin oxidase could be required(15). As a result, formazan salts are generated that are insoluble in water. A solvent such as Dimethyl sulfoxide (DMSO) can dissolve this formazan salt. The formazan product is evaluated at 595nm via spectroscopy and its absorbance is related to the number of live cells(16).

MTT assay was performed to assess cell viability in logarithmically establishing HeLa cells treated with varied concentrations of piperine for 24 h and 48 h. A comparison of piperine's cytotoxic effect on HeLa cervical cancer cells at

various concentrations demonstrates that cell survival can be reduced to 93, 87, 86, 82, and 17%, at concentrations of 62.5, 125, 250, 500, and 1,000 µg/mL, respectively. At the same concentration, piperine could lower cell viability to 31; 55; 57; 67; and 77% after 48 hours of incubation. These findings offer concrete proof that piperine selectively and dose-dependently kills cervical cancer cells. Piperine significantly reduced the cell viability in a time- and dose-dependent manner.

The cytotoxic effect of piperine on HeLa cervical cancer cells was examined in this study using IC_{50}, a metric that indicates the concentration at which 50% of the cells growth is inhibited. The IC_{50} value indicates how hazardous the substance is, and the higher it is, the less toxic it is. A linear regression equation for the correlation between concentration and percentage of cell viability can be used for estimating the IC_{50} value. The amount of cancer cells that persist is directly correlated with the strength of the purple hue that forms. HeLa cells were used in the piperine cytotoxic test, and the results indicated that an IC_{50} value obtained after incubation for 24 and 48 hours, were 711.21 µg/mL and 419.75 µg/mL, respectively. The United States National Cancer Institute characterizes cytotoxic activity as having an IC50 value of 20 g/ml, a moderate cytotoxic activity of 21-200 g/ml, a weak cytotoxic activity of 201-500 g/ml, and no cytotoxic activity of >500 g/ml. This studi shows piperine had no cytotoxic effect after 24 hours, however after 48 hours, the cytotoxic activity increased to weak cytotoxicity(17).

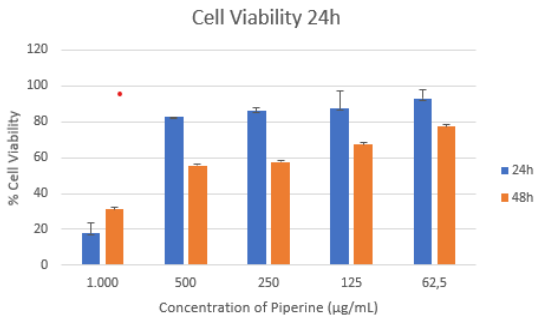

Figure 2. HeLa cell viability after being treated by Piperine with various concentration after 24h and 48 treatment

The fundamental mechanism of the test is also unknown. There are still inconsistencies and unknowns about certain aspects, such as what other organelles, enzymes, and molecules play a role in MTT reduction, the formation of extracellular formazan crystals, the cytotoxic impact of the MTT reagent itself, and whether the assay evaluations indicate cell viability, activity of metabolism, and therapy toxicity(18).

Many studies have attempted to uncover piperine's primary chemopreventive mechanism of action, which includes apoptosis signaling cascade activation, inhibition of cell proliferation, cell cycle arrest, changes in redox homeostasis, ER modulation stress and autophagy, angiogenesis inhibition, induction detoxification enzymes, and tumor sensitization to radiotherapy and chemotherapy. The mode as mentioned earlier of action of piperine suggests that it may play an essential role in cancer chemoprevention (19).

CONCLUSION

Piperine has been reported to have an effect on cell viability. A decreasing cell viability value is indicated by an increase in piperine concentration. The duration of piperine treatment has a direct effect on its cytotoxic efficacy against HeLa cells. After 48 hours of treatment, the percentage of viable cells significantly decreased. This indicates that the time and concentration of piperine treatment affect its cytotoxic effects on HeLa cells.

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CONFLICT OF INTERESTS (if any)

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