The Screening Of Cytotoxic Fraction From *Elephantopus scaber* Linn against Human Cervical Cancer (Hela) Cells

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Abstract

Purpose: Cervical cancer has become the second leading cause of death after breast cancer. *Elephantopus scaber* Linn (ES) has been used traditionally for curing various diseases. The objective of this study was to explore the active fraction from ES as anticancer and the mode of cell death. **Method**: The ES herb were extracted using maceration method with ethanol followed by evaporation to get the concentrated extract. The extract were fractionated using petroleum ether, chloroform, ethyl acetate and methanol respectively. The cytotoxic activity of were carried out with MTT method, and the mode of cell death were observed by acridine orange-Ethidium bromide double staining. **Result:** The result showed that the ES fraction has cytotoxic activity against HeLa cell lines with IC_{50} values of petroleum ether, chloroform, ethyl acetate and methanol fractions were 185; 42.26; 95.72; 650 µg/ml respectively. The mode of cell death showed by the doublestaining method were apoptosis. The cell dead could be distinguish from live cells. The cell dead appear to absorb the ethidium bromide as the DNA of the cell membrane was damage and ethidium bromide could interact with DNA of the cells, while the live cells do not absorb the ethidium bromide as the cell membrane still intact. **Conclusion**: The chloroform fraction of ES is the most cytotoxic fraction against HeLa cells.

Keywords: Elephantopus scaber, cytotoxic, IC_{50}, apoptosis.

INTRODUCTION

Cancer is the second leading cause of death after cardiovascular disease. Cervical cancer is the most frequently cause of cancer death in women especially in developing countries. The cervical cancer therapy has developed and progressed rapidly, but the mortality and the incidence of cervical cancer is still high. The treatment of cancer is consist of surgery, radiotherapy, chemotherapy and hormone therapy. Surgery cannot be performed at metastatic stage of cancer. Chemotherapy could be an alternative for controlling cancer. Nevertheless, despite showing good results, chemotherapy has many side effects and high toxicity [1].

The failure of chemotherapy can be associated with the failure of anticancer agents to induce programmed cell death (apoptosis) [2]. Resistance of cancer cells to chemotherapy are widely reported, it can be caused by overexpression of PGP in cells that lead to the presence of drug efflux out of the cell. Therefore, development of new cytotoxic agents for cancer therapy is urgently needed [3].

Chemotherapeutic agents currently in use are cytotoxic and affect both normal and malignant cells. Side effects include bone marrow suppression, nausea and vomiting, epilation, renal, cardiac and neurotoxicity [4]. This prompted scientists to search for new compounds that are more effective with minimum toxic effects.

Some research has been reported the potential effect of *Elephantopus scaber* Linn (ES) as anticancer agent. The ES extract was proven to induce apoptosis against cervical cancer [5,6], breast cancer cells [7, 8]. Some chemical constituent has been identified as antitumor were deoxyelephantopin [9], scabertopinol, trans-caffeic acid, methyl 3,4-dicaffeoylquinate, luteolin-41-O-β-D-glucoside, trans-p-coumaric acid, indole-3-carbaldehyde, methyl trans-caffeate, luteolin-7-O- glucuronide 6"-methyl ester, and luteolin [10]. The objective of this study was screen the active fraction against human cervical carcinoma (Hela) cells for further development as herbal product for cervical cancer therapy.

MATERIAL AND METHOD

Collection of plant material

The plant material was collected from Magetan, East Java. The plant material was dried and grind.

Extraction and Fractionation

The 200 g of ES powder were extracted using maceration method with 900 ml of ethanol, the extract were collected and evaporated to get the concentrated extract. The concentrated extract were dissolved to the petroleum ether and shaked for 6 hours and allowed to equilibrium for 24 hrs. The soluble fraction were

separated as petroleum ether fraction and the nonsoluble fraction were then fractionated using chloforom, ethyl acetate and methanol respectively. All fraction were evaporated and the dried fraction were collected.

Cytotoxicity test

Cytotoxicity assay was performed using MTT method. The 100 mL suspension of HeLa cells at a density of $1x10^4$ were incubated in a CO_2 incubator with 100 mL RPMI medium and ES fraction with concentration series of test 2000; 1500; 1000; 800; 400; 200; 100; 50; 25; 12.5; 6:25; and 3.125ug / ml.

After 24 h incubation at 37 ° C (5% CO_2) in 96 wells microplate, media was discarded by inverting the plate slowly. After media disposed add 100 mL of MTT, then incubated for 4 hours at 37 ° C, 5% CO_2. After incubation, the mixture were added with 100 mL of SDS solution in 0.01 N HCl and incubated for over night at room temperature. The microplate were read by ELISA reader at 550 nm wavelength. The IC_{50} values were then calculated from percentage of living cells.

Acridine orange-Ethidium bromide double staining

HeLa cells were grown on the coverslip placed onto 24 wells microplate with density of $2x10^4$ cells/ wells, and incubate at 37 °C for 24 hours. Subsequently, cells were added with samples at different concentration and incubate for 24 hours. After incubation medium were washed with PBS. Furthermore, coverslip was taken and placed on an object glass. The 10 uL solution of acridine orange and ethidium bromide were added and then allowed to stand for 10 minutes. The apoptotic cells were observed using fluorescence microscope with 400x magnification.

RESULT AND DISCUSSION

Medicinal plants are considered a repository of numerous types of bioactive compounds possessing varied therapeutic properties. The therapeutic potential of plants has been well explored over a very long time period. Cancer is one of the major problems to human health around the world. Among all epidemic diseases, cancer holds the first place as a death- causing disease. The great potential of plant-based compounds for the treatment and prevention of cancer is attributed to their safety, low cost, and oral bioavailability. Cytotoxic screening of a number of plants has been done to correlate their anticancer activity and further expand their scope for drug development.

Elephantopus scaber Linn, have been used traditionally to treat various diseases as antiinflammation, diarrhea, hepatitis, arthritis [11]. With the diverse traditional applications of *E. scaber*, United Nations Development Program has recommended *E. scaber* as a potential natural herb which should be further studied [11].

Cytotoxicity assay

The cytotoxicity test against Hela cell lines were used as parameter to screen the potential activity of ES fraction against cervical cancer cells. The MTT method was used to evaluate the potential cytotoxicity [12]. The MTT method was based on the reduction of tetrazolium salt by the NADH enzyme of live cells. The tetrazolium salt were detected by spectrophotometric method. Figure 1 showed the percentage of Hela cell viability after ES fraction treatment. The viability of Hela cells were showed in dose dependent manner. The cytotoxicity of ES fractions were performed in Table I.

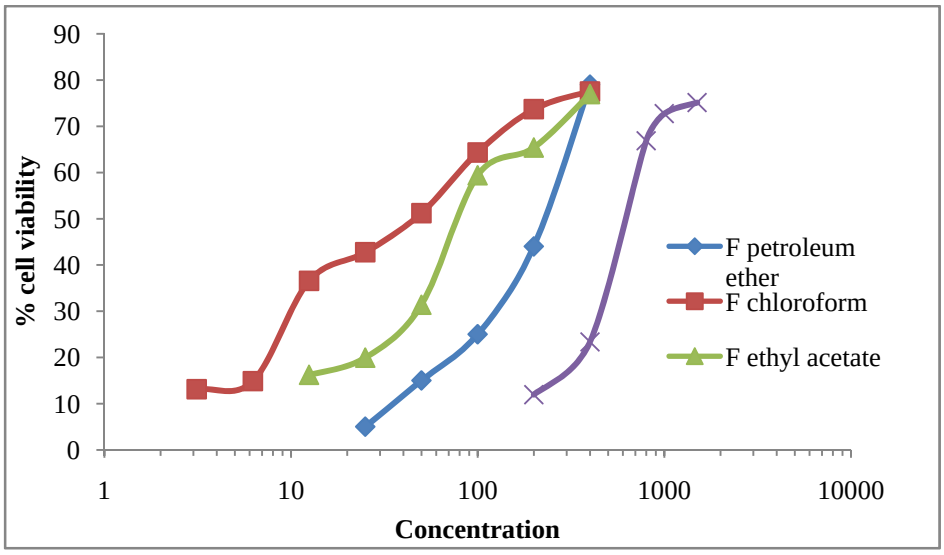

Figure 1. The percentage of HeLa cell viability after ES fraction treatment. The number of cell viability showed in dosedependent manner.

Table I Cytotoxicity of ES fractions against Hela cells

Fraction	IC50
Petroleum ether fraction	184,59 ug/ml
Chloroform fraction	46,266 µg/ml
Ethyl acetate fraction	95,72 ug/ml
Methanol fraction	650,35 µg/ml

The result showed that chloroform fraction and ethyl acetate fraction has IC50 lower than 100 µg/ml, it showed that this fractions potential to develop as anticancer agent. This fraction may be rich with active compound. This faction became the most active fraction may be due to the highly content of active ingredients. Chloroform and ethyl acetate may be a suitable solvent to dissolve the active substance.

Some sesquiterpen lactone has been has been isolated and reported as a potential anticancer agent. Isodeoxyelephantopin, deoxyelephantopin has been isolated as active compound through bioassayguided fractionation [13]. These compound has a lower polarity and may be isolated in high content using relatively nonpolar solvent (chloroform fraction).

Apoptosis inducing effect

The mode of killing that is induced by most anticancer agents is by apoptotic cell death. The antiproliferative activity shown by ES fraction could be possibly due to the induction of apoptosis. To determine the mode of growth inhibition in HeLa cells induced by ES fraction, the acridine orange-ethidium bromide doublestaining were carried out. HeLa cells were treated with a dose equivalent of an, IC_{50} of ES fractions. The cell undergoing apoptosis display a profound destruction of the nucleus that results in the formation of nuclear blebs containing DNA. Staining of apoptotic cells with a fluorescent DNA-binding dye allows for easy detection of this phenomenon

After treated with ES fractions for 24 h, HeLa cells showed active apoptosis. The apoptotic cells showed the red florescense of ethidium bromide as the rupture of cell membrane. Etidium bromide is the DNA dye could enter the nucleus and interact with DNA. The apoptotic cells could be differentiated with live cells. The live cells displayed the green florescence caused by absorption of acridine orange, but the etidium bromide cannot enter in the nucleus as the membrane integrity in the healthy cells.

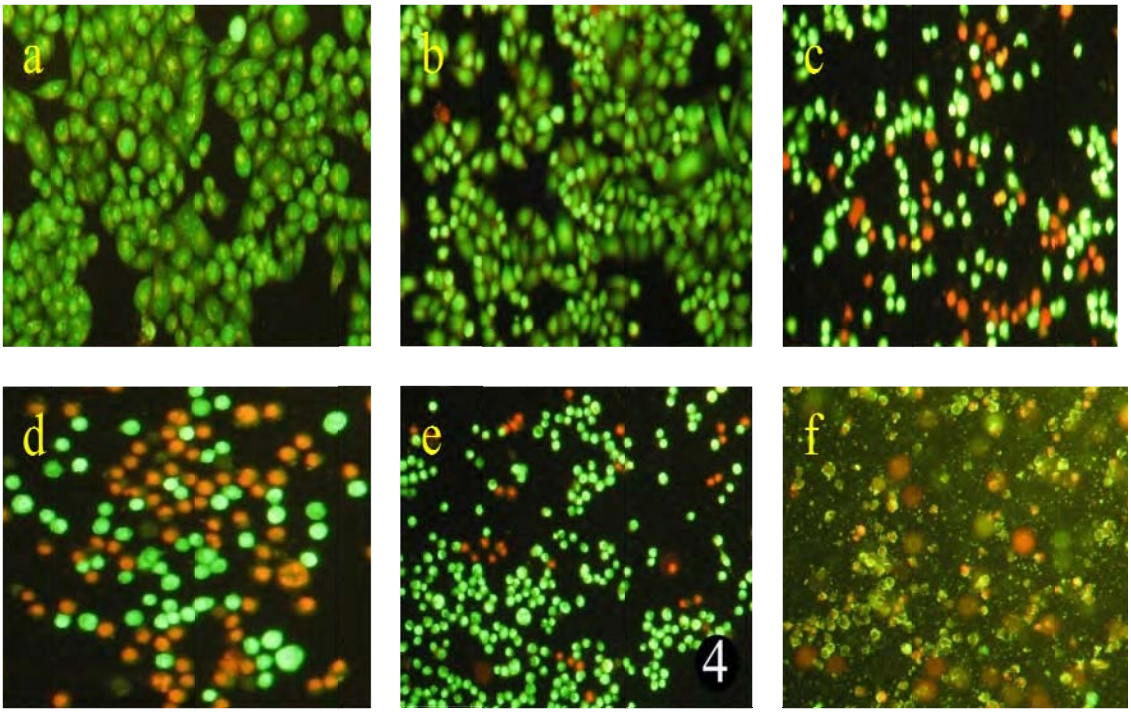

Figure 2. The AO-EtBr doublestaining of ES-fraction treated HeLa cells, a. control cell, b. DMSO-treated cell, c. Petroleum ether fraction of 185µg/ml, d. chloroform fraction of 46,26 µg/ml, e. ethyl acetate fraction of 96 µg/ml, f. methanol fraction of 650 µg/ml.

This study found that ES fraction induce apoptosis against Hela cells. Isodeoxyelephantopin, a sesquiterpen lactone isolated from chloroform extract of *Elephantopus scaber* was reported to induce apoptosis in nasopharyngeal epidermoid (KB) cancer cells [14]. The chloroform fraction was found to be the most cytotoxic fraction and potential to be developed as herbal anticancer product.

CONCLUSIONS

The chloroform fraction of *Elephantopus scaber* is the most actife fraction against Hela cells. The mode of celldeath induce by *Elephantopus scaber* fraction is apoptosis.

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