Cytotoxic activity of Buah Merah fractions
(Pandanus conoideus Lam) towards cervical cancer cell in
HeLa Cells Culture

Hana Ratnawati*, Wahyu Widowati, Diana K. Jasaputra, Sylvia Soeng
Medical Research Centre
Medical Faculty, Maranatha Christian University, Bandung
Jl. Prof.drg. Suria Sumantri 65 Bandung 40164-Indonesia
*e-mail: hanna_ratnawati@yahoo.com

Abstract

Cervical cancer is the most common type of cancer in Indonesian women. Buah Merah is Indonesia natural plant and promising anticancer activity according empiric data. This research was done to examine the cytotoxic activity of four fractions (first, second, fourth, and sixth fraction) of Buah Merah (Pandanus conoideus Lam) towards cervical cancer cell in HeLa cells culture. Buah Merah fractions were examine in various level and compared with doxorubicin as positive control. The results showed that the second fraction of Buah Merah has the most potentially dose (0.5 and 1 µl/ml) to inhibit the growth of HeLa Cells. It might be concluded that fraction of Red Fruit has cytotoxic activity towards cervical cancer cell in HeLa cell culture.

Keywords: Buah Merah (Pandanus conoideus Lam), HeLa cell, cytotoxic, cervical cancer

Introduction

Cervical cancer is the most common cancers that affect Indonesian women and the second common female cancer in the world. About 99.7 % of cervical cancer associated with human papilloma virus (HPV) infection. The main oncogenic strains are types 16 and 18. Estimates suggest that up to 75% of all sexually active people will infect at some point in their lives. In a small group of women, the virus survives for years before it eventually converts some cells on the surface of the cervix into cancer cells. In the majority of cases, the infection does not cause any symptoms, but in some women, HPV infection can progress the development of cervical intraepithelial neoplasia, which can lead precancerous and cancerous lesions of the uterine cervix (U.S. Cancer Statistics Working Group, 2007; Cancer-msnbc.com. 2008; Wikipedia).

All women are at risk for cervical cancer. It occurs most often in women aged 30 years and older. In 2004, in the United States 11,892 women had cervical cancer, and 3,850 died from cervical cancer (U.S. Cancer Statistics Working Group, 2007). Half of cervical cancer cases occur in women between ages 35 and 55. Every year more than 10,000 women in the United States are diagnosed with invasive cervical cancer, and nearly 4,000 die of cervical cancer (MayoClinic.com, 2006). Each year an estimated 500,000 women are diagnosed with the disease and about 300,000 die from it, mostly in the developing world (Cancer-msnbc.com. 2008). Each year between 2,700 new cases cervical cancer in the UK, only one percent of new cancer cases diagnosed. About 1,000 women per year die of cervical cancer in the UK (Wikipedia, 2008). Worldwide, cervical cancer is the fifth most deadly cancer in women. It affects about 1 per 123 women per year and kills about 9 per 100,000 per year (WHO, 2006; Wikipedia, 2008). Worldwide it is estimated that there are 473,000 – 500,000 cases of cervical cancer, and 253,500 deaths per year (Wikipedia, 2008; Healthcommunities.com, 2008).

Based on the data from 13 Indonesia Patology Centre, cervical cancer cases achieved 28.7 %. The incidence of cervical cancer at Dharmais Cancer Hospital in 1995 – 2002 was 1259, in 2003 – 2004 the incidence was 402 patients. Based on the data from National Health Department, there was 90 – 100 new cervical cancer cases per 100,000 women. Every year an estimated 200,000 new cases cervical cancer was detected in Indonesia (Bestantia Indraswati, 2005).

The higher risk for developing cervical cancer is women who have had many sexual partners and or began having sexual intercourse at an early age and any condition that weakens immune system’s, include HIV, having had an organ transplantation, hormonal contraception, multiple pregnancies and a family history of cervical cancer. Smokers are at least twice as likely as non-smokers to develop cervical cancer. Women have a poor diet and are infected HPV, the body may be less able to fight off the virus, so more cells may undergo the genetic changes that can lead to pre-cancerous cells and then to cervical cancer. (Cancer Research UK, 2007; Dolinsky and Hill-Kasyer).
About 80-90% of cervical cancers are squamous cell carcinomas, occurring in the flat squamous cells that cover the outside of the cervix. Most other cases are adenocarcinomas, rising from mucus-producing gland cells of the inner endocervix, adenosquamous carcinoma, small cell carcinoma, neuroendocrine carcinoma. A few cervical cancers are mixtures of these types (Cancer Research UK, 2005; Wikipedia, 2008). Approximately 10-15% of cases develop in glandular surface cells called adenocarcinomas (Healthcommunities.com, 2008).

The sign and symptoms of cervical cancer are: 1). Vaginal bleeding after sexual intercourse, between regular menstrual periods or after menopause; 2). Watery, bloody vaginal discharge that may be heavy and have a foul odor, thick, or contain mucus; 3). Pelvic pain or pain during intercourse 4). Pain in urination; 5). Blood in the stool or urine ((MayoClinic.com, 2006; Dolinsky and Hill-Kasyer, 2008; Fayed, 2008). Symptoms of advanced cervical cancer may include: loss of appetite, weight loss, fatigue, pelvic pain, back pain, leg pain, single swollen leg, heavy bleeding from the vagina, leaking of urine or feces from the vagina, and bone fractures (Wikipedia, 2008).

Cervical cancer usually takes a very long time for pre-cancerous lesions to progress to invasive cancers and women have effective screening methods that can detect pre-cancerous lesions generally be cured without serious side effects. Regular screening with a Pap smear effectively lowers the risk for developing invasive cervical cancer by detecting precancerous changes in cervical cells (Dolinsky and Hill-Kasyer, 2008).

Many ways treating cervical cancer, include surgery, radiotherapy, chemotherapy. Surgery is generally only treated in early stage cervical cancer. Higher stage disease is usually treated with radiotherapy and chemotherapy (Dolinsky and Hill-Kasyer, 2008; Wikipedia, 2008). Chemotherapy have many negative side effects, due to most of chemotherapy drug contain synthetic compound. Very important to explore natural herbal with cytotoxic activity.

Indonesia have many original plants with anticancer activity, such as Buah Merah which empirically use for cancer treatment and inhibit the progression of cancer.

Human immortal cancer cell lines have constituted an accessible, easily usable set of biological models with which to investigate cancer biology and to explore the potential efficacy of anticancer drugs. HeLa cell is one of the cells grown from the cervical cancer of a young African-American woman, Henrietta Lacks. HeLa cells were the first human cells to be continuously grown in culture. The cells were first cultured in February 1951 by Drs. George and Margaret Gey at Johns Hopkins in Baltimore. The cells appear “immortal” and are still used in medical research today. (Webster's New World Medical Dictionary, 2008).

**Materials and Method**

**Materials**

Flacon, conical tube15 ml & 50 ml, hemocytometer, Inverted microscope, CO2 incubator, Laminar Air Flow Cabinet, centrifuge, micropipette, microwell plate 96, tissue culture flask

**Chemicals**

HeLa cell line (from Laboratorium Ilmu Hayati UGM), RPMI 1640, trypan blue. Fetal Bovine Serum (FBS) 70%, Fungizone, Penicilline, Streptomycine, Trypsine, doxorubicin.

**Plant material**

Sample of Buah Merah (*P. conoidues* Lam) were collected from Papua Indonesia

**Extraction and fractionation**

The flash of fruit without seed (5 kg) of *P. conoidues* Lam were soaked in MeOH and evaporated resulted crude extract. The methanol crude extract was partitioned with *n*-hexane and ethyl acetate contained 5 % ethyl acetate, continued with *n*-hexane and ethyl acetate contained 50 % ethyl acetate, continued with *n*-hexane and ethyl acetate contained 66 % ethyl acetate, continued with ethyl acetate 100 %, continued with ethyl acetate and methanol contained 50 % methanol, continued with methanol 100 %. Qualitative evaluation base on retention factor (Rf). First fraction with Rf value 0,58; second fraction Rf was 0,36; four fraction Rf was 0,12 and sixth fraction Rf was 0,56.

Preparation serial concentration of Buah Merah fraction and doxorubicin (as positive control) with 2 level concentration: 0,5 µl/ml and 1 µl/ml.

**Research Preparation:**

HeLa cell line is used for anticancer assay.

1. Grow the Hela cells in a flask with 7 ml medium (*composition*: 500 ml DMEM; 10% final Fetal Bovine Serum; 1/100 final Streptomycine/Penicilline. Put in the CO2 incubator. Change the medium every 2 days. When the cells are at about 90 % confluence, pour out the medium and apply Trypsine 0,25% for 30 min at 37°C.

2. Verify under microscope that all cells are detached from the flask; transfer the suspension in a 15 ml Falcon tube and centrifuge for 10 min at 2000 rpm. Aspirate the supernatant (be careful not to aspirate the cell pellet).

3. Re-suspend the pellet in 1 ml RPMI, pipette 20 µl (cell)+180 µl (trypan blue) put in the Hemocytometer and count, under an optical microscope, the number of cell within the square.

4. Put 12 ml RPMI into the pellet and pour 100 ul to each well plate and put the microwell plate in the CO2 incubator.
Table 1 The anticancer activity of fractions and doxorubisin (%)

<table>
<thead>
<tr>
<th>Cytotoxic substrate:</th>
<th>Concentration (µl/mL)</th>
<th>1</th>
<th>0.5</th>
</tr>
</thead>
<tbody>
<tr>
<td>BM Fraction I</td>
<td></td>
<td>47.384</td>
<td>40.743 a</td>
</tr>
<tr>
<td>BM Fraction II</td>
<td></td>
<td>100.000 g</td>
<td>100.000 g</td>
</tr>
<tr>
<td>BM Fraction IV</td>
<td></td>
<td>97.625 f</td>
<td>89.107 de</td>
</tr>
<tr>
<td>BM Fraction VI</td>
<td></td>
<td>50.214 e</td>
<td>46.445 b</td>
</tr>
<tr>
<td>Doxorubisin</td>
<td></td>
<td>91.002 e</td>
<td>86.947 d</td>
</tr>
</tbody>
</table>

The same letter show no significant at the 5 % (Duncan’s test)

Figure 1 HeLa cell culture which added by BM fraction 1 µl/ml

Anticancer or cytotoxic assay
Take out the well plate from the incubator and into each well which already contain 100 µL culture cell (36,000 – 37,000 live cell), then added by:
- 100 µl Buah Merah fractions with 2 level concentration 0.5 µl/ml and 1 µl/ml (duplo)
- in another well plate added 100 ul doxorubicin as positive control
- incubated at CO₂ incubator for 24 hours
- count the live cell using Cell Counting Direct with trypan blue and continued calculated the anticancer activity using the formula:

% death cell = \( \frac{\sum \text{live cell control} - \sum \text{live cell treatment}}{\sum \text{live cell of control}} \times 100 \)

Results and Discussion

The cytotoxic activity toward HeLa cell line of four fractions of Buah Merah (P. conoidaes Lam) of 2 level concentration were measured to know the capability fractions to damage and kill cancer cell culture. The cytotoxic activity fractions of Buah Merah is shown in Table 1. The cytotoxic activity of fractions indicated that the second fraction showed the strongest cytotoxic effect, it was 100 % at concentration 1 µl/mL and 0.5 µl/mL, fourth fraction showed stronger than first and sixth fraction also compared to doxorubisin as positive control. Second and fourth fractions of Buah Merah are effective to decrease cancer proliferation of HeLa cells.

Conclusions

Second fraction Buah Merah and fourth fraction Buah Merah with 0.5 and 1µl/ml are a promising anticancer toward cervical cancer in the HeLa cell culture.

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