INTERRODUCTION

A powder from dried flesh fruit of Phaleria macrocarpa (Scheff) boerl has been used as medicinal purpose and reportedly remedy of disentry, diabetes mellitus, hypertension, cancer, as a cure for some skin deseases, etc (1). Many researches have been done to know the fruit effect as an anticancer, but it effect to normal cells haven’t been done yet.

Former research showed ethanol extract of Phaleria macrocarpa (scheff) boerl flesh fruit has the highest toxicity to HeLa cell line derived from cervix carcinoma. The aim of this research was 1) to know the toxicity of the ethanol extract of Phaleria macrocarpa (scheff) boerl fruit to normal cell, 2) to know the effect of ethanol extract of Phaleria macrocarpa (scheff) boerl flesh fruit to p53 and Bcl-2 gene expression. So, after we knew the effect to normal cell, we could use the Phaleria macrocarpa (scheff) boerl fruit as cancer remedy more safely.

The content of Phaleria macrocarpa (scheff) boerl flesh fruit essential oil were octadeca, tricosan, octacosen, diochromylester and tributhylacethylcitrat, while the seed consist of heptadeca, octadeca, eicosan, tricosan, vinyl laurat, diochromylester, flavonoide, triterpenoide and coumarin groups, while the seed consist of alkaloide, terpenoido and coumarin groups (3). Besides, n-hexane extract of Phaleria macrocarpa (scheff) boerl flesh fruit contained terpenoid compound, whereas the seed contained steriode (4). The ethanol extract of Phaleria macrocarpa (scheff) boerl seed and fruit meat were not toxic to human mononuclear perifer normal cell, but slightly toxic to vero cell line. Tamoxifen and 5-fluorourasil were very toxic to mononuclear perifer normal cell and vero cell line. The ethanol extract of Phaleria macrocarpa (Scheff) boerl seed has higher cytotoxicity than fruit meat extract. The imunohistochemistry result explained that the ethanol extract of Phaleria macrocarpa (Scheff) boerl seed and fruit meat and tamoxifen did not increase p53 and decrease Bcl-2 genes expression. There was a possibility that the dying cell mechanism was caused by necrosis, not by apoptosis.

KEYWORDS: anticancer, Phaleria macrocarpa, cytotoxicity

ABSTRACT

There were many research on Phaleria macrocarpa (Scheff) boerl fruit for its activity as anticancer. However, there was none investigation on Phaleria macrocarpa (Scheff) boerl seed and fruit meat extract of ethanol effect against normal cell. The study conducted to identify the cytotoxicity of ethanol extract of Phaleria macrocarpa (Scheff) boerl seed and fruit meat extract of ethanol effect against normal cell. The study conducted to identify the cytotoxicity of ethanol extract of Phaleria macrocarpa (Scheff) boerl seed and fruit meat extract of ethanol effect against normal cell. Besides, It was also investigated the p53 and Bcl-2 genes expression. The research consist of several sections including Phaleria macrocarpa (Scheff) boerl seed and fruit meat maceration with ethanol, cytotoxicity test to mononuclear perifer normal cell and vero cell line, and imunohistochemistry test to p53 and Bcl-2 genes expression. As positive control was used Tamoxifen dan 5-fluorourasil. The result showed that the ethanol extract of Phaleria macrocarpa (Scheff) boerl seed and fruit meat were not toxic to human mononuclear perifer normal cell, but slightly toxic to vero cell line. Tamoxifen and 5-fluorourasil were very toxic to mononuclear perifer normal cell and vero cell line. The ethanol extract of Phaleria macrocarpa (Scheff) boerl seed has higher cytotoxicity than fruit meat extract. The imunohistochemistry result explained that the ethanol extract of Phaleria macrocarpa (Scheff) boerl seed and fruit meat and tamoxifen did not increase p53 and decrease Bcl-2 genes expression. There was a possibility that the dying cell mechanism was caused by necrosis, not by apoptosis.

Keywords: anticancer, Phaleria macrocarpa, cytotoxicity

INTRODUCTION

The research showed that chloroform extract of Phaleria macrocarpa (scheff) boerl leaf had toxicity to Artemia salina Leach Larva with LC₅₀ 2,722 ± 0,027 μg/mL, while the flesh fruit had LC₅₀ 3,218 ± 0,147 μg/mL (5), and the seed had LC₅₀ sebesar 5,113.10⁻⁵ ± 3,213.10⁻⁵ μg/mL (6).

The ethanol extract of Phaleria macrocarpa (scheff) boerl seed fruit had toxicity to T47D breast cancer cell line through COX-2 expression inhibition. The toxicity activity possessed LC₅₀ 15,12 ± 3,21 μg/mL (7). Besides, the ethanol extract of seed and flesh fruit increased the p53 gene expression but no effect to Bcl-2 gene expression (8) and the n-hexane extract of seed had the higher effect to increase the p53 gene expression than flesh fruit, but no effect to Bcl-2 gene expression.

Apoptosis is the most common form of eukaryotic cell death. It is a physiological suicide mechanism that preserves homeo-stasis, in which cell death naturally occurs during tissue turnover. In general, cells...
undergoing apoptosis display profound structural changes, including a rapid blebbing of the plasma membrane and nuclear disintegration. The nuclear collapse is associated with extensive damage to chromatin and DNA cleavage into oligonucleosomal-length DNA fragments after activation of calcium-dependent endogenous endo-nucleases. Apoptosis is essential in many physiological processes, including the embryonic development and the maturation of the immune system. It is currently the subject of intense research, partially because we now recognise that tumor cells are susceptible to death by apoptosis in response to drugs and/or radiation treatment.

The majority of in vivo studies suggest that the inhibitory effect of p21 is largely exerted during the G1 phase of the cell cycle, with preferential binding to Cdk4- and Cdk2-containing complexes, and that it either inhibits their kinase activities or prevents their activation by CAK. The regulation of p21 is largely dependent on the presence of functional p53, a transcriptional regulator that mediates cell cycle arrest following DNA damage and in senescence. So, the cell cycle will be ended and cell turn into apoptosis. These effects probably play a role in the ability of p53 to act as a tumor suppressor. In addition, a number of chemopreventive agents have been shown to exert their anti-tumorigenic activity through p53-dependent mechanisms. Bcl-2, Bclx, Bax, Bad, Bik are protein that involved in apoptosis. Bcl-2 group (Bcl-2 and Bcl-xl) is a protein that inhibit apoptosis. Overexpression of Bcl-2 protein restrain apoptosis initiation by c-myc, so the genes were involved in tumorigenesis (9, 10, 11).

MATERIALS AND METHODS

Material
- Phaleria macrocarpa (scheff) boerl from Beringharjo Market
- Tamoxifen 10 mg (Tamofen ®), 5-Fluorouracil “Ebewe” 250 mg
- Vero cell line from LPPT UGM
- Human mononuclear perifer normal cell from donor

Methods
Determination of Cytotoxic effect.
The Vero cell line was cultured in RPMI 1640 medium with 10% fetal bovine serum (FBS) (especially for T47D using fetal calf serum (FCS)) and 1% penicillin/streptomycin in humidified incubator (37°C, 5% CO2). Cell culture medium and supplements were obtained from Gibco Life Technologies. Human mononuclear normal cell from donor was used directly.

Cytotoxicity assays were conducted in 96 well plate ELISA plate (Nunc). Cells were treated with various concentrations of ethanol extracts of Phaleria macrocarpa (scheff) boerl. Each well received 100 μL of cell suspension with cells density 5x10⁴/100 μL and 100 μL of the appropriate dilution of extract. As a control, cells were held in the medium without added of sample. Then, plates were incubated in a 37°C humidified incubator in 5% CO2 for 24 hour. After cultivation for 24 hours, the cytotoxic effect was examined by MTT reagent. There were 3 replicate wells for each treatment. Tamoxifen and 5-Fluorouracil used as positive controls were done by the same manner.

Imunocytochemistry
Cells at the density 10⁴/100 μL were cultured by adding sample in IC₅₀ concentration and grown in a humidified incubator at 37°C under 5% CO₂ in air for 24 hours. After incubation, cells were collected and washed in PBS. They were, then resuspended in RPMI, placed in object glass, fixation by acetone for 5 minutes, incubated in hydrogen peroxide for 10-15 minutes and then wash again with PBS. Cells were added by p53 or Bcl-2 monoclonal antibody (primary antibody), incubated for at least 1 hour and then washed four times with PBS. Secondary antibody Biotinylated Goat Anti-Polyvalent was added to cell, incubated at room temperature for 10 minutes and washed four times with PBS. DAB (chromogen) was put in to cell, incubated for 3-8 minutes, washed with aquadest. Then, the cell put in to hematocilyn solution for 3-4 minutes. The cells were observed under microscop, cells expressed the gene p53 or Bcl-2 if the cell had brown.

RESULT AND DISCUSSION

Toxicity of Seed and Flesh Fruit of Phaleria macrocarpa (scheff) boerl
The induction of the cells death was characterized by the changing of the cells morphology and the cells viability for treated and untreated cells. On the treated cancer cells, density of cells and percentage of cell death were higher compared to the untreated cells. The higher of the added extract the higher of the normal cells death. Figure 1 was shown the toxicity of Phaleria macrocarpa (scheff) boerl seed and flesh fruit ethanol extract to vero cell line and human mononuclear normal cell. As indicated on figure 1, the ethanol extract had higher toxicity to vero cell line than human mononuclear normal cell. At the 900 mg/mL concentration, the two extracts gave 76% inhibition to vero cell line, and 25% inhibition to human mononuclear normal cell by ethanol extract of flesh fruit and 45% inhibition by seed.

Tamoxifen and 5-Fluorourasil were cancer remedy used as positive control. Figure 1 was representing the toxicity of tamoxifen and 5-Fluorourasil to vero cell line and human mononuclear
Figure 1. Vero cell line and human mononuclear cell % inhibition by ethanol extract of *Phaleria macrocarpa* (scheff) boerl flesh and seed fruit.

Tamoxifen and 5-FU were more toxic to vero cell line compared to human mononuclear cell, while tamoxifen had higher toxicity to both cells compared to 5-FU. This difference in the cytotoxic level seemed to be caused by the differences in the mechanism to cancer cells. 5-FU is a pyrimidin antagonism and has to undergo anabolisms to active compound 5-fluoro-2’deoxyuridin 5’-monophosphat. The active compound will affect DNA synthesis on early phase to thymineless by tymidilat syntetase inhibition. The cell will die because of thymineless death. Thus, 5-FU act depend on 5-FU alteration to active compound effectively. While, tamoxifen take action as estrogen synthesis inhibitor directly. Tamoxifen mechanism based on it ability to compete with estradiol binding to estrogen receptor. So, it will decrease receptor total in cytoplasm. The effect made tumor cell growing inhibition because tumor cell growing depend on estrogen.

To compare the toxicity between ethanol extract of *Phaleria macrocarpa* (scheff) boerl flesh, seed fruit, tamoxifen and 5-FU were used IC50 (Inhibition Concentration 50) value (table 1). The IC50 showed that ethanol extract of *Phaleria macrocarpa* (scheff) boerl seed fruit had higher toxicity than flesh fruit. This differences on the cytotoxic level seemed to be caused by more anticancer active compound in seed than flesh fruit. Tamoxifen and 5-FU were very toxic to normal cell compared to ethanol extract of *Phaleria macrocarpa* (scheff) boerl seed and flesh fruit.

The ethanol extract of *Phaleria macrocarpa* (scheff) boerl seed had toxicity value IC50 15,12 ± 3,21 μg/mL to breast cancer cell line (T47D) (7). Therefore, Based on the IC50 value, ethanol extract of *Phaleria macrocarpa* (scheff) boerl seed and flesh fruit were not toxic to human mononuclear cell but slightly toxic to vero cell line. But, tamoxifen and 5-FU had no selectivity to cancer cell because very toxic to normal cell too.

![Figure 1](image1.png)

![Figure 2](image2.png)

![Figure 3](image3.png)

Table 1. IC50 value

<table>
<thead>
<tr>
<th>Cell</th>
<th>Flesh</th>
<th>Seed</th>
<th>Tamoxifen</th>
<th>5-Fluorourasil</th>
</tr>
</thead>
<tbody>
<tr>
<td>Vero</td>
<td>241,99</td>
<td>75,35</td>
<td>0,32</td>
<td>1,06</td>
</tr>
<tr>
<td>Mononuclear</td>
<td>3977,29</td>
<td>1181,03</td>
<td>3,07</td>
<td>3,13</td>
</tr>
</tbody>
</table>

Endang Astuti, et al.
Figure 3 showed that there was no difference on \( p53 \) gene expression between untreated and treatment cell. Untreated Vero cell line gave blue cells, while treatment cell gave the blue cell too. Thus, the ethanol extract of *Phaleria macrocarpa* (scheff) boerl seed and flesh fruit and tamoxifen didn’t increase \( p53 \) gene expression.

There was no difference on \( bcl-2 \) gene expression between untreated and treatment cell (figure 3). Untreated Vero cell line gave brown cells, while treatment cell gave the brown cell too. Thus, the ethanol extract of *Phaleria macrocarpa* (scheff) boerl seed and flesh fruit and tamoxifen didn’t decrease \( bcl-2 \) gene expression. Based on the fact, the extracts and tamoxifen made apoptotic inhibition. This was appropriate with the fact that the extracts and tamoxifen didn’t increase \( p53 \) gene expression.

**CONCLUSION**

1. Ethanol extract of *Phaleria macrocarpa* (scheff) boerl seed and flesh fruit were not toxic to human mononuclear cell but slightly toxic to vero cell line.
2. Tamoxifen and 5-FU had no selectivity to cancer cell because very toxic to normal cell too.
3. The ethanol extract of *Phaleria macrocarpa* (scheff) boerl seed and flesh fruit and tamoxifen didn’t increase \( p53 \) and decrease \( bcl-2 \) gene expression

**REFERENCES**