Cell Cycle Inhibition from Ethylacetate Extracts of *Plectranthus amboinicus* (Lour.) Spreng.) Leaves on HeLa Cells Lines

Rosidah¹, Poppy Anjelisa Z. Hasibuan¹, Denny Satria²

¹ Departement of Pharmacology, Faculty of Pharmacy, University of Sumatera Utara, Medan, 20155, Indonesia
² Departement of Pharmaceutical Biology, Faculty of Pharmacy, University of Sumatera Utara, Medan, 20155, Indonesia

correspondence author: poppyanjelisa94@gmail.com

ABSTRACT

Objective: To evaluate the effects of ethylacetate extract (EAE) of *Plectranthus amboinicus* (Lour.) Spreng.) leaves on cell cycle on HeLa cell lines.

Methods: Analysis of cell cycle distribution was performed using flowcytometer and the data was analyzed using ModFit LT 3.0 program.

Results: The EAE changes the accumulation of cell cycle phase from G0-G1 phase (54.61%) to sub-G1 phase (69.73%).

Conclusions: Based on the results, EAE is potential to be developed as co-chemotherapeutic for cervics cancer by cell cycle arrest. However, the molecular mechanism needs to be explored further.

Keywords: cell cycle, *Plectranthus amboinicus* (Lour) Spreng., HeLa, sub G1.

Background

Cancer is one of the major causes of death in many countries. Effective anticancer drug with selectivity against only malignant cells and with ability to repress tumor metastasis are desired. Research into plants with anticancer effects is still encouraged with a view to discover any new drugs with less toxic but more potent effects. *Plectranthus amboinicus* (Lour) Spreng., (*Coleus amboinicus*, Lour., *Coleus aromaticus*, Benth.), or ‘daun bangun-bangun’ is one of plant used as lactagogue by native people in north Sumatera, Indonesia. This plant has also been traditionally used for the treatment of inflammation, heart disease, as diuretic, immunomodulator and hepatoprotector. Phytomedicines derived from plants have shown great promise in the treatment of cancer disease. Recent studies have revealed that anticancer activity of *Plectranthus amboinicus* (Lour) Spreng. extracts on MCF7 and T47D may be related to the inhibition of cell cycle and induced cell death.

The aim of this study are to evaluate the cytotoxic effects of ethylacetate extract (EADBB) of *Plectranthus amboinicus* (Lour.) Spreng.) leaves on HeLa cell lines and to evaluate the effects of ethylacetate extract (EADBB) of *Plectranthus amboinicus* (Lour.) Spreng.) leaves on cell cycle on HeLa cell lines.
Materials and Methods

1. Plant Material
Plectranthus amboinicus was obtained from Pematang Siantar, North Sumatera. The leaves were dried at 45°C and ground into powder. The dried leaves powder extracted with ethylacetate by maceration method.

Analysis of cell cycle distribution was performed using flowcytometer and the data was analyzed using ModFit LT 3.0 program.

2. Cytotoxicity assay
Cytotoxicity was determined by the MTT assay.

Briefly, HeLa cells were plated at $10^4$ cells/well in a 96-well plate. After incubation for 24 h at 37°C, cells were treated by ethylacetate extract of ‘daun bangun-bangun’ (EADBB) with different concentration and incubated for 24 h. MTT solution was added to each well and further incubated for 4 h at 37°C, optical density was read with an ELISA reader at 595 nm.

3. Flowcytometry assay
Cell cycle inhibition assay
- HeLa cells ($5 \times 10^5$ cells/well) were seeded into 6-well plate and incubated for 24 h.
- The cells were treated with extract, and then incubated for 24 h. Both floating and adherent cells were collected in conical tube using tripsin 0.025%.
- The cells were washed thrice with cold PBS and centrifuged 2500 rpm for 5 min. The supernatant was separated, while the sediment was collected and fixed in cold 70% ethanol in PBS at -20°C for 2 h.
- The cells were washed thrice with cold PBS and resuspended then centrifuged 3000 rpm for 3 min and PI kit (containing PI 40 µg/mL and RNAse 100 µg/mL) added to sediment and resuspended and incubated at 37°C for 30 min.
- The samples were analysed using FACScan flowcytometer. Based on DNA content, percentage of cells in each of stage in cell cycle (G1, S and G2/M) were calculated using ModFit Lt. 3.0.s.

Results
The treatment of EADBB was showed the inhibition of cells growth. The IC$_{50}$ value of EADBB 143.291 µg/mL and doxorubicin 1.8 µg/mL. The effect of EADBB treatments is given in Figure 1-4. Whereas, single treatment of doxorubicin on ½ IC$_{50}$ and IC$_{50}$ caused cell accumulation at sub G$_1$ phase (37.55% and 24.62%). Treatment of EADBB on ½, 1x dan 2x IC$_{50}$ caused cell accumulation at sub G$_1$ (68.59%; 69.73%; 68.61%).

Table 1. HeLa cell distribution after treatments with various concentrations of EADBB and doxorubicine

<table>
<thead>
<tr>
<th>Treatments</th>
<th>concentration</th>
<th>Sub G$_1$</th>
<th>G$_0$-G$_1$</th>
<th>S</th>
<th>G$_2$–M</th>
</tr>
</thead>
<tbody>
<tr>
<td>control</td>
<td>-</td>
<td>12.19</td>
<td>54.61</td>
<td>6.09</td>
<td>9.67</td>
</tr>
<tr>
<td>EADBB</td>
<td>1/2 IC$_{50}$</td>
<td>68.9</td>
<td>15.18</td>
<td>6.99</td>
<td>3.14</td>
</tr>
<tr>
<td>EADBB</td>
<td>1 IC$_{50}$</td>
<td>69.73</td>
<td>12.26</td>
<td>6.38</td>
<td>3.23</td>
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<tr>
<td>EADBB</td>
<td>2 IC$_{50}$</td>
<td>68.61</td>
<td>14.74</td>
<td>6.93</td>
<td>3.02</td>
</tr>
<tr>
<td></td>
<td>½ IC&lt;sub&gt;50&lt;/sub&gt;</td>
<td>27.62</td>
<td>21.23</td>
<td>6.69</td>
<td></td>
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</tr>
<tr>
<td>Doxorubicine</td>
<td>50</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Doxorubicine</td>
<td>1 IC&lt;sub&gt;50&lt;/sub&gt;</td>
<td>24.62</td>
<td>19.82</td>
<td>14.69</td>
<td></td>
</tr>
</tbody>
</table>

Figure 1. Cell cycle analysis using flowcytometry

The phytochemical analysis of Plectranthus amboinicus, (Lour.) Spreng. Revealed the presence of phenols and flavonoids (Hasibuan, et al., 2013) and the cytotoxic activity of the leaf is attributed to the presence of these compounds. Flavonoid reduce breast cancer and may also cervical cancer proliferation, by inhibiting cell growth, protein kinase activities, and induction apoptosis (Vargas and Burd, 2010). Phenolic compounds inhibit different cell cycles arrest at different cell phases and have different effect on cell cycle arrest and subsequently induce apoptosis (Mahadev, et al., 2015).

**Conclusion**

The EADBB showed slight cytotoxic effect on HeLa cell lines. The analysis of cell cycle showed cells undergo apoptosis, showed by occurrence of apoptosis during inhibition of cell cycle on sub-G<sub>1</sub> phase.

**References**


