

Arcangelisia flava LEAVES ETHANOLIC EXTRACT SUPPRESSES CANCER CELL LINES VIA NON APOPTOTIC PATHWAY

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INTRODUCTION

Arcangelisia flava is a potential candidate to be developed as cancer chemoprevention agent. *A. flava* was proven to exhibit antioxidant and cytotoxic activity against MCF-7 breast cancer cell line. These ability were suggested to be related to its alkaloid content: berberine, palmatine, and jatrorrhizine (Keawpradub *et al.*, 2005). Although this plant is stated as a rarely found species (Koran Jakarta, 2012), we could find it abundantly in Meru Betiri National Park, Jember.

Our previous studies showed that *A. flava* leaves increase the immune system in doxorubicin-treated rats (Puspitasari *et al.*, 2014^b) with no signs of toxicity based on sub chronic toxicity assay (Puspitasari *et al.*, 2014^a). The *A. flava* leaves ethanolic extract (EEAFL) had been proven to have cytotoxic activity on HeLa, MCF-7, and WiDr cancer cell lines with the IC₅₀ value of 467 ± 70; 136 ± 17; and 213 ± 79 µg/ml, respectively. The activity was selective on MCF-7 and WiDr, but not likely on HeLa cell line (Puspitasari *et al.*, 2015).

OBJECTIVES

This study was conducted to determine whether the cytotoxic activity of EEAFL was occur via apoptotic or necrotic pathway using flowcytometry annexin V-FITC method. The concentration used for the assay were concentration approx. the IC₅₀ and the IC₇₅ based on previous study (Puspitasari *et al.*, 2015).

MATERIAL AND METHOD

Plant Materials and Extraction

The leaves were obtained from Meru Betiri National Park, Jember, Indonesia without any selection on age, only for their health and freshness. The ethanol extract was prepared based on Puspitasari *et al.* (2015). The leaves were washed and air-dried, then they were grounded and sieved. The ethanolic extract were prepared using 100 g of leaves powder. The ground-dried leaves was extracted with ethanol 96%. The extraction was repeated three times. The ethanol extract was evaporated under reduced pressure (Heidolph, Laborota) resulting EEAFL. EEAFL

was then suspended in DMSO never exceed than 1% for flowcytometry annexin V-FITC apoptosis assay.

Flowcytometry Annexin V-FITC Apoptosis Assay

The flowcytometry annexin V-FITC apoptosis assay were done on HeLa cervical cancer cell line, MCF-7 breast cancer cell line, and WiDr colon cancer cell line. All of the cancer cell lines were the collection of Parasitology Laboratory, Faculty of Medicine, Gadjah Mada University.

Cancer cell lines (5x10⁴ cells/well) were seeded in 6 well plate and incubated using suitable media for 24H in 37°C 5 % CO₂ for the adaptation. HeLa and WiDr were grown in RPMI 1640, while MCF-7 was grown in DMEM. The media used was supplemented with 10% of fetal bovine serum, 1% of penicillin-streptomycin, and 0.5% fungizone.

EEAFL was then given to the cell line at approx. IC₅₀ and IC₇₅ (concentration that inhibits 50% and 75% of cells), respectively. The apoptosis assay was done for 24H, except on HeLa was done for 48H. At the end of the incubation time, the cell line was harvested using trypsin and washed using cold PBS twice. The cell line, then, was centrifuged and added 100 µl annexin V-FITC diluted in binding buffer containing propidium iodide (50 µg/ml). The cell line was incubated further for 15 min at room temperature before analyzed with flowcytometer (FACSCalibur Becton Dickinson) at λ 488 nm.

Data was obtained in the form of living cells, apoptotic cells, and likely undergo necrotic cells distribution. The analysis was done based on annexin V and propidium iodide fluorescence intensity. Annexin V would identified the flip out cell membrane at the apoptotic cells (phosphatidylserine directs to the outside of the cell). While propidium iodide would detect the exposed DNA either due to the cell membrane breakage during the the apoptotic bodies formation as well as necrosis.

RESULTS AND DISCUSSION

The EEAFL obtained was 16.1 g from 100 g ground-dried leaves, resulting the yield of 16.1%. The flowcytometry annexin V-FITC apoptosis assay

showed that the method was valid, since more than 90% of the cell control was alive (see Fig. 1, Fig. 2,

and Fig. 3).

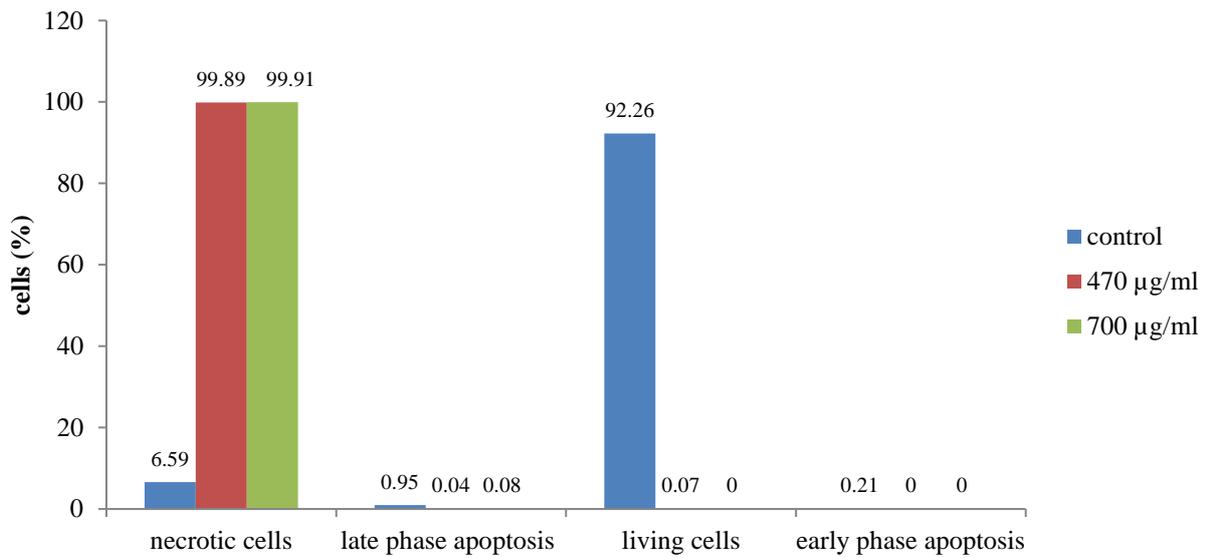


Fig. 1. The flowcytometry annexin V-FITC apoptosis assay of HeLa cell line treated with EEAfL for 48H. EEAfL treatment at IC₅₀ and IC₇₅ cause necrosis rather than apoptosis in HeLa cell line.

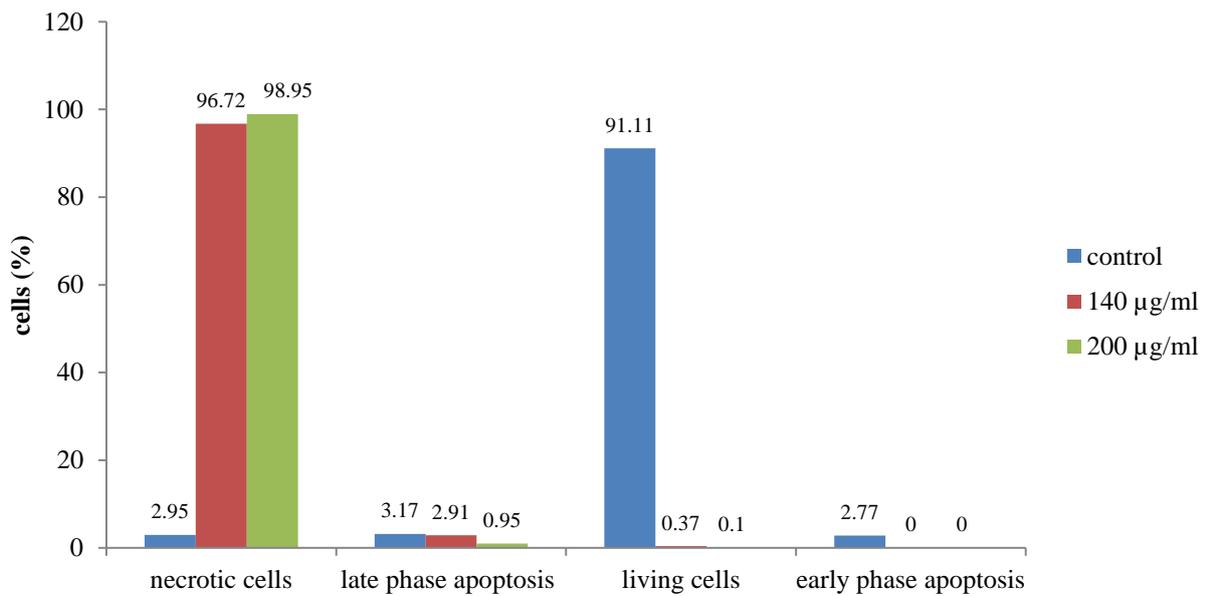


Fig. 2. The flowcytometry annexin V-FITC apoptosis assay of MCF-7 cell line treated with EEAfL for 24H. EEAfL treatment at IC₅₀ and IC₇₅ cause necrosis rather than apoptosis in MCF-7 cell line.

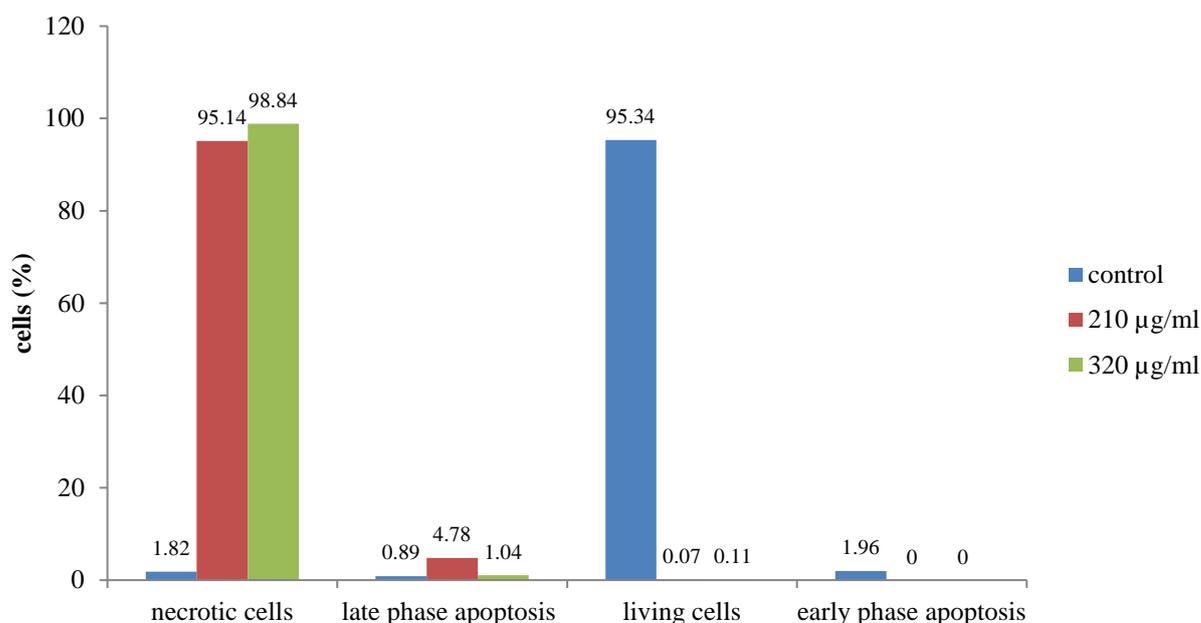


Fig. 3. The flowcytometry annexin V-FITC apoptosis assay of WiDr cell line treated with EEAfL for 48H. EEAfL treatment at IC₅₀ and IC₇₅ cause necrosis rather than apoptosis in WiDr cell line.

EEAfl treatment at approx. IC₅₀ and IC₇₅ were likely to trigger necrosis rather than apoptosis on HeLa, MCF-7, and WiDr. The higher the EEAfl concentration pushed the more cells to undergo necrosis (Fig. 1, Fig. 2, and Fig. 3), except on HeLa cell line, the effect of approx. IC₅₀ and IC₇₅ was the same (Fig. 1).

Necrosis is a cell death process that is avoided in cancer therapy. It would cause inflammation at the cancer microenvironment. The pro-inflammatory agent released would further generate malignant growth of cancer itself, resulting worse prognosis for the patients (Hanahan and Weinberg, 2011).

The necrosis activated by the EEAfl was suggested due to the high concentration used. Though IC₅₀ was supposed to induced apoptosis than that of the IC₇₅ (Mahassni and Al-Reemi, 2013), of course it would correlate to the compounds content in EEAfl. EEAfl contains alkaloids, flavonoids, terpenoids, and saponin (Maryani *et al.*, 2013). Alkaloids may cause apoptosis as well as necrosis on cancer cell line (Lamchouri *et al.* 2013; Song *et al.*, 2006), while most flavonoids are inducing apoptosis rather than necrosis (Kuno *et al.*, 2012). The effect might be less harmful if the concentration used is lower, e.g. the using of EEAfl in combination with cancer chemotherapeutic agent (co-chemotherapy). Yet, studies have to be taken to support this theory.

CONCLUSION

EEAfl tends to trigger necrosis rather than apoptosis on HeLa, MCF-7, nor WiDr. Still, further research on combinatorial use of EEAfl and cancer chemotherapeutic agent is needed to reveal whether EEAfl is better use as cancer co-chemotherapy rather than single use.

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