Cell Cycle Arrest Activity of Alkaloid Fraction of Litsea Cubeba Lour. Heartwoods Towards Hela Cancer Cell

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Abstract: Cervical cancer therapy with chemotherapeutic agents is limited because of drug resistance problem and toxic effect on normal tissue leads to immunosuppression and cardiotoxicity. This study was to investigated cell cycle arrest activity towards HeLa cell lines of Litsea cubeba Lour. heartwood alkaloid fraction. Litsea cubeba Lour. heartwood powder was extracted by maceration method with ethanol 96% and fractionated with n-hexane and chloroform at pH 3,7 and 9. The cytotoxic study was using MTT method and analysis cell cycle was using flow cytometry method. The IC50 of ethanol extract, n-hexane and chloroform fractions at pH 3,7 and 9 at were 156.24 ± 2.96; 67.23 ± 0.63; 175.92 ± 2.40; 52.46 ± 0.34; and 94.81 ± 2.16 µg/mL respectively. The chloroform fractions at pH 7 concentration 25 and 10 µg/mL were caused accumulation in G2-M phase (33.84 and 29.08%). The results reveal that Litsea cubeba Lour. heartwood alkaloid fraction provides effective as cell cycle arrest. Our further study is to assess the mechanism of alkaloid fraction in inhibit metastasis in cervical cancer.

1 INTRODUCTION

Cancer is one of the high incidence dangerous diseases in human and presently there is a considerable number of new anticancer agents from natural products (Sharma, et al., 2011). According to WHO data, cancer is one of the leading cause of death worldwide especially cervical cancer (Berrington and Lall, 2012). Cervical cancer theraphy with chemotherapeutic agents is limited cause of drug resistance and toxic side effect on normal tissue leads to some effects such as immunosuppression and cardiotoxicity (Jemal, et al., 2010; Tyagi, et al., 2004).

Attarasa (*Litsea cubeba* (Lour,) is a plant from Lauraceae family which contain many essential oils which used as antideppressants, antiinflammation, antioxidant, pesticide, antimicrobial, anticancer on breast cancer and neuro pharmacology. The methanol extract from attarasa fruits showed to be active on HeLa cell lines which cause apoptosis through activation of caspase 3/7 (Trisonthi, et al., 2014; Piyapat, et al., 2013). There are more than forty isoquinoline alkaloids that contained in *Litsea* genus which are active as antibacterial agents against *Staphylococcus aureus* (Feng, et al., 2009). The heartwoods of *Litsea cubeba* contained high level of phenolic and flavonoid and found to be active as antioxidant and has anti breast cancer activity which causes cell cycle inhibition. Alkaloids compound which isolated from heartwood have antioxidant activity with DPPH and ABTS methods (Dalimunthe, et al., 2016; Dalimunthe, et

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al., 2017; Dalimunthe, et al., 2018). The aim of this study was to assess cell cycle arrest activity of alkaloid fraction of *Litsea cubeba* Lour. heartwoods on HeLa cells.

2 MATERIALS AND METHODS

2.1 Fractions Preparation

Fresh heartwoods of *Litsea cubeba* Lour. was collected from Balige subdistrict, Sumatera Utara province, Indonesia. The air-dried and powdered heartwoods of *Litsea cubeba* (Lour,) (1 kg) were repeatedly macerated with ethanol 96% (3x3 d, 7.5 L), The filtrate was evaporated to give a viscous extract. Viscous extract was fractionated with n-hexane and continue with chloroform at pH 3,7 and 9 (Rosidah, et al., 2018; Dalimunthe, et al., 2018; Satria, et al., 2015).

2.2 Cytotoxicity assay

Extract and alkaloid fractions were submitted for cytotoxicity test. In that way, HeLa cell line was grown in RPMI medium containing 10% Fetal Bovine Serum (Gibco), 1% penicillin-streptomycine (Gibco), and fungizone 0.5% (Gibco) in a flask in a humidified atmosphere (5% CO_2) at 37ºC. The inoculums seeded at 1 x 10^4 cells/mL at an optimal volume of 0.1 mL per well. After 24 h incubation, the medium was discharged and treated by fractions. After incubation for 24 h, the cells were incubated with 0.5 mg/mL MTT for 4 h at 37ºC. Viable cells reacted with MTT to produce purple formazan crystals. After 4 h, SDS 10% as stopper (Sigma) in 0.01N HCl (Merck) was added to dissolve the formazan crystals. The cells were incubated for 24 h in room temperature and protected from light. After incubation, the cells were shaken, and absorbance was measured using microplate reader at λ 595 nm. The data which were absorbed from each well were converted to percentage of viable cells (Hasibuan, et al., 2015 and Nurrochmad, et al., 2014).

2.3 Cell Cycle Inhibition Assay

HeLa cells ($7.5x10^5$ cells/well) were seeded into 6-well plate and incubated for 24 h. After that, the cells were treated and then incubated for 24 h. Both floating and adherent cells were collected in conical tube using trypsin 0.025%. The cells were washed thrice with cold PBS and centrifuged at 2500 rpm for 5 min. The supernatant was separated, while the sediment was collected and fixed in cold 70% ethanol in PBS at 4ºC for 1 h. The cells were washed thrice with cold PBS and resuspended then centrifuged at 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 37ºC for 30 min. The samples were analyzed using FACScan flow cytometer. 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 (Harahap, et al., 2018 and Satria, et al., 2017).

2.5 Statistical Analysis

The results were presented as means ± SD.

2.5.1 Results

Inhibitory Concentration 50% (IC_{50})

MTT method was used to determine cell viability after incubation for 24 h. In every treatment extract and alkaloid fractions were shown in Table 1.

Table 1. IC_{50} value of extract and alkaloid fractions of *Litsea cubeba* heartwood with MTT assay (Mean ± SD, 3 times of replication)

Treatment	IC_{50} (µg/mL)
Ethanol Extract	156.24 ± 2.96
n-hexane Fraction	67.23 ± 0.63
Chloroform Fraction pH 3	175.92 ± 2.40
Chloroform Fraction pH 7	52.46 ± 0.34
Chloroform Fraction pH 9	94.81 ± 2.16
Cisplatin	24.01 ± 0.31

2.4 Effect on Cell Cycle

To evaluate the effect of chloroform fraction at pH 7 (CF-7) to increase cell death by modulating cell cycle, we concentrated on it for further studies using flow cytometry method.

The effect of CF-7 at 25 and 10 µg/mL is given in Figure 1. Whereas treatment of CF-7 at 25 and 10 µg/mL caused cell accumulation at G_2/M phase (33.84% and 29.08%) and for control cell (17.78%).

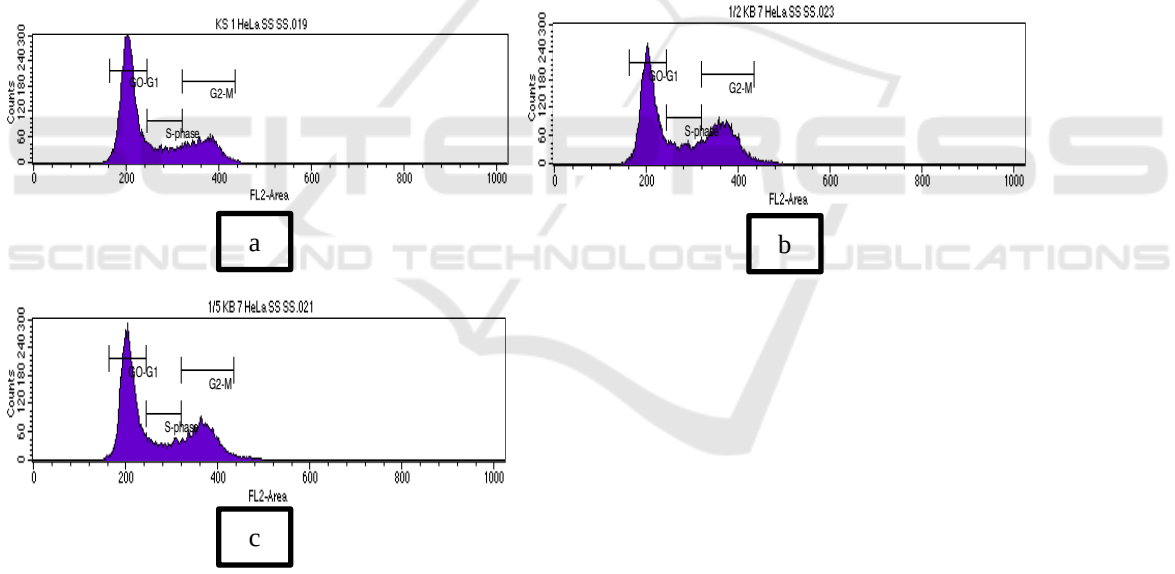

Figure 2: Percentage of cell cycle phase of HeLa cells were treated for 24h. (a) Control cell, (b) 25 µg/mL (1/2 IC_{50}), (c) 10 µg/mL (1/5 IC_{50}).

3 DISCUSSION

The cytotoxicity estimate of herbal is correlated to content of active compound in these plants including *Litsea cubeba* Lour. Alkaloids as major compound have main role in cytotoxicity effect (Yadav, et al., 2010). *Litse*a genus is rich in isoquinoline alkaloids and for *Litsea cubeba* Lour has been found two alkaloids (+)-*N*-(methoxy-carbonyl) *N*-norlauroscholtzine and (+)-*N*-(methoxy-carbonyl)-*N*-norglaucine (Feng, et al., 2009). Alkaloids are the compound which potentially in inhibits the cancer proliferation for the example berberine is an isoquinoline alkaloid which inhibits proliferation of multiple cancer cell line by inducing cell cycle arrest at G_0/G_1 or G_2/M phases and by apoptosis (Sun, et al., 2009; Eom, et al., 2010; Burgeiro, et al., 2011). Inhibition of tumor invasion and metastasis is the mechanism of action of berberine (Tang, et al., 2009; Ho, et al., 2009). Evodiamine is a quinolone alkaloid inhibits topoisomerase enzyme, induces DNA damage, exhibit G_2/M phase arrest (Liao, et al., 2005; Kan, et al., 2004; Huang, et al., 2004).

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