Anti-migration Effect of *Aaptos suberitoides* Fraction in HCT-116 Colorectal Cancer Cell Line

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Abstract

Background: Colorectal cancer is the second leading cause of mortality and the most prevalent cancer worldwide. Most patients, who come with late-stage, have ineffective treatments and some side effects in chemotherapy. *Aaptos suberitoides* has potential anti-cancer effects due to its bioactive compounds such as aptamine. This study aimed to evaluate the migration inhibition effect of *Aaptos suberitoides* fraction in HCT-116 cell line.

Methods: This study was an experimental study. *Aaptos suberitoides* specimen was taken in Tinjil Island and fractionated with ethyl acetate. HCT-116 cell line was added with *Aaptos suberitoides* fraction and cellular migration activity was observed in 48 hours of which the scratch assay was performed. The gap closure area was determined with ImageJ software.

Results: The data showed that a low concentration of *Aaptos suberitoides* fraction inhibited migration activity in HCT-116 cell line as follow; 1 and 5 mg/L Aaptos suberitoides fraction inhibit 3-4 % cancer cell migration in 24 hours, and 10-11% inhibition in 48 hours, respectively. However, 10 mg/L fraction concentration only inhibited 7-14% of the migration effect.

Conclusions: *Aaptos suberitoides* fraction suggests insignificant migration inhibition in colorectal cancer cells and only inhibits less than 15 % HCT-116 cell line.

Keywords: *Aaptos suberitoides*, HCT-116, scratch assay

Introduction

Cancer is one of the non-communicable diseases that ranks second in mortality rate worldwide of which colorectal cancer is the second most common prevalent according to the World Health Organization (WHO) data in 2018.[1] Nowadays, colorectal cancer treatments have a good prognosis when it is treated in early-phase. Unfortunately, most patients come in a late-phase conditions leading to ineffective treatment.[2] Late-phase treatment may also lead to unwanted side effects and complications. Moreover, chemotherapy causes weakness, shortness of breath, diarrhea, constipation, and even heart attack.[3,4] Therefore, patients need more effective treatment in suppressing cancer activity to increase life expectancy and inhibit metastasis.

Indonesia is an archipelagic country with a sea covering a two-third area of the country. The ocean has high marine biodiversity and marine biota is known to have a good impact on health, amongst many of them is sea sponge. Sea sponge apparently can be utilized for cancer treatment.[5] An example of this is eribulin, a Halichondria okadai derivate that has been used for the treatment of breast cancer and liposarcoma metastatis.[6] Another sea sponge that has potency as an anti-cancer agent is *Aaptos suberitoides*. One active compound, Aaptamine, has been known with invitro to have anti-cancer activity.[7] The *Aaptos suberitoides* extract can inhibit 20–40% migration activity in HCT-116.[8] In the HCC-1954 cell line, this extract (5 mg/l) reveals 45.23% gap closure in 72 hours.[9] Moreover, this extract (5 mg/l) has a potent effect in MDA-MB 231 TNBC cell line.[10]

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To prove that *Aaptos suberitoides* has an anti-cancer effect especially in colorectal cell line migration, we had conducted *Aaptos suberitoides* anti-migration potency in HCT-116 cell line.

Methods

This was an experimental study conducted in cell culture and cytogenic laboratory Universitas Padjadjaran, in august 2020. *Aaptos suberitoides* specimen was collected from Tinjil Island and fractionated by liquid-liquid extraction method in aquades, n-hexane, and ethyl acetate solvent. The ethyl acetate fraction, which was the most potent in those fractions was diluted with dimethylsulfoxide (DMSO) 100%. Fraction concentration in this research was set as 1 mg/l, 5 mg/l, and 10 mg/l based on IC50 of *Aaptos suberitoides* maceration extract for HCT-116 as published previously.[8]

This research used HCT-116 as a cell line. The cell line was cultured in Roswell Park Memorial Institute (RPMI) medium with additional supplementation of 10% fetal bovine serum (FBS) and 1% Penstrep in culture cell incubator, setin a controlled temperature at 37°C and CO^2 concentration in 5%. The cell was seeded in 12 well plates using different concentrations that were 0,1,5, and 10 mg/l with three replication for every treatment set in one timetrial.

When measuring in vitro cells, a migration scratch assay was performed. First, this cell line was seeded in 24 hours. Then the medium was changed with another medium in which FBS concentration was 1% to induce cellular starvation. This condition was needed to decrease bias when observing cell proliferation.

After 18 hours, the medium was then replaced again by a medium with 10% FBS concentration. The sample was then scratched and washed with phosphate buffered saline (PBS). The gap area was captured by a microscope at 0,24, and 48 hours.

Gap closure area was performed by 100% - (cleft treatment area in 24 or 48 hours/cleft treatment area in 0 hour x 100%). Migration inhibition rate wass performed by (treated gap closure area (1 or 5 or 10 mg/l)/controlled gap closure area (0 mg/l)) -100%.

The gap closure area was analyzed using ImageJ software. The gap closure against control were calculated with Excel (Microsoft, USA) software and was presented in percentage. Shapiro-Wilk test was performed as normality testing. One-way ANOVA with posthoc analysis was performed as hypothesis testing and was considered statistically significant if $p < 0.05$.

Results

After cellular starvation, HCT-116 cell line

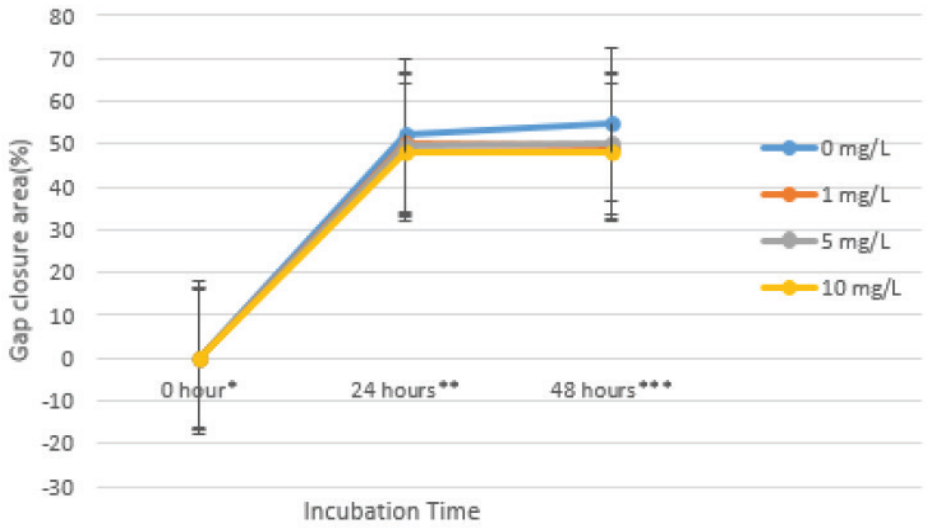

Figure 1 Mean Gap Closure Area of HCT-116 Treated with *Aaptos suberitoides* Fraction (0, 1, 5, and 10 mg/l) for 0,24 and 48 hours

Note: *P = 0.84 **P = 0.84 ***P = 1.1

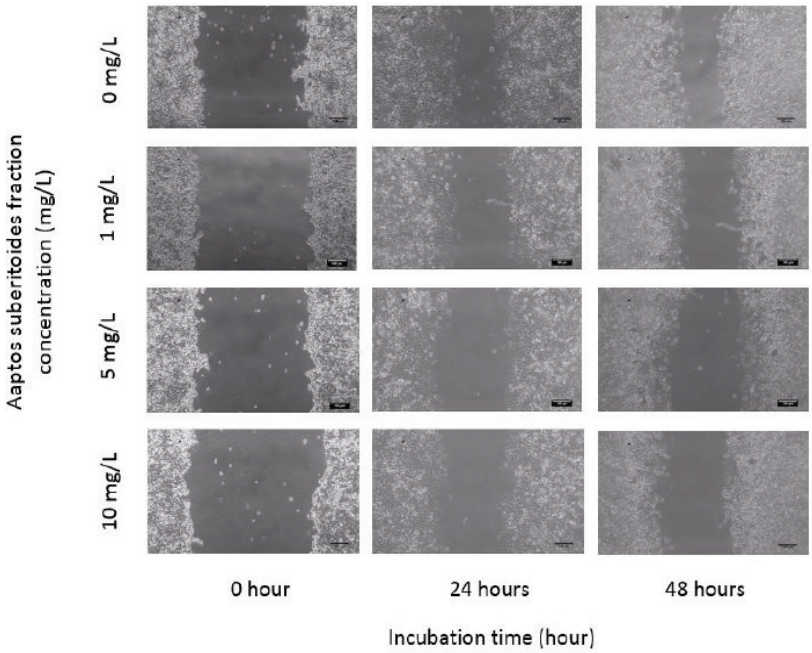

Figure 2 Microscopic Pictures from Scratch Assay in Control (0 mg/l) and Treated Cell (1, 5, and 10 mg/L) for 0, 24, 48 Hours Incubation

was given with Aaptos suberitoides fraction as treatment in low concentration until 48 hours. Our data showed that treated cells had lower migration activity than control cells. The normality test showed that the data had a normal distribution. One-way ANOVA analysis showed that the p-value in 24 hours was 0.84 and thep-value in 48 hours was 1.13 ($p > 0.05$) (Figure 1). Based on the gap closure area, in cells treated with 1 and 5 mg/l *Aaptos suberitoides* fraction concentration, migration inhibition occurred 3–5% in 24 hours and 10–11% in 48 hours, respectively.

Then in cells treated with 10 mg/l *Aaptos suberitoides* fraction concentration, the migration inhibition is observed in 7% after 24 hours and 14% in 48 hours, respectively. The microscopic pictures were shown in Figure 2.

Discussion

The cellular gap closure in our study indicates a statistically insignificant result, although the data seems in normal variances. These results can not prove that low concentration fraction (1, 5, and 10 mg/l) have a significant migration-inhibition impact. Theoretically, *Aaptos suberitoides* contains alkaloid compound such as aaptamine, isoaaptamine, and dimethyleaaptamine which have an important role in migration inhibition.[11] Based on biochemical activity, Aaptamine can increase cofilin-1 concentration.[12] Cofilin-1 detach F-actin filament by using the actin-depolymerizing factor (ADF), then it inhibits actin-myosin movement.[13] Increase of Cofilin-1 expression also inhibit Matrix Metalloprotein (MMP) activity, especially MMP-1, MMP-3, MMP-9.[14] Moreover, MMP inhibition is affected with Activator Protein-1(AP-1) and Nuclear Factor-κB (NF-κB) activity too. Aaptamine can deactivate Mitogen-Activated Protein Kinase (MAPK) and AP-1. Additionally, Aaptamine also decreases proinflammatory cytokine expressions such as cyclooxygenase-2, tumor necrosis factor-alfa, interleukin-1 beta, and NF-κB. Consequently, AP-1 and NF-κB inhibition also can inhibit MMP expression.[15]

Our data has an insignificant result. After 24 hours, gap closure activity in the treatment cell almost reaches 50% near the control. This condition can occur because of the increase of MMP activity after cellular starvation. This increase of MMP activity can also directly increase the migration activity higher than the migration-inhibition effect.[16] The purpose of cellular starvation is to alleviate proliferation bias in this research. Cellular starvation inhibits cellular proliferation and induces cell cycle arrest.[17] However, the MMP activity as a side effect may be higher than the migration-inhibition effect of *Aaptos suberitoides* fraction in 1, 5, and 10 mg/l.

This research has some limitations. This

research trial set only one-time trials without positive control. This condition can impact data significance. Further study needs to be conducted using positive control. Furthermore, we can not eliminate Aaptos suberitoides that has the anti-migration effect, since other studies have proved otherwise with supporting result.[8,9,10] To evaluate this research, method evaluation is needed. Using other migration assays like Boyden Chamber assay and ORIS may affect the result. Other bias elimination methods like starvation setting and mitomycin C treatment can be considered.

In conclusion, *Aaptos suberitoides* fraction in this study has insignificant anti-migration effect on HCT-116 cell line. The further explorative study is imperative to show the anti-migration effect of Aaptos suberitoides

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