ORIGINAL ARTICLE

Viability Test of α-Mangostin against Oral Squamous Cell Carcinoma

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ABSTRACT

Introduction: Oral Squamous Cell Carcinoma (OSCC) represents approximately 96% of the entire oral cancers. Epithelial-mesenchymal transition (EMT) is a factor contributing to the poor prognosis associated with OSCC. α-mangostin is one of the xanthones which show anti-cancer activities against some types of cancers and can suppress EMT-induced invasion by increasing E-cadherin expression. This study aimed to identify the viability of α-Mangostin to reduce the viable cells of HOC313. **Methods:** The role of α-mangostin to induce HOC313 cell culture at various concentrations which conducted on two groups: control group using only HOC313 cell line and intervention group comprising HOC313 cell line which added various concentrations. In this present study, cells were treated after reaching the confluency level of 80% in 5x103 cells/well. α-mangostin used had six concentrations: 1.25 µM, 2.5 µM, 3.75 µM, 5 µM, 6.25 µM, and 7.5 µM. **Results:** Concentration of α-mangostin had a significant effect on cell viability, p-value obtained was at 0.023 ($p < 0.05$). The Mann-Whitney test was also performed to identify significant differences in cell viability between control cells and all treatment cells were at 2.5 mg/ml and 5 mg/ml with the value $p = 0.02$ ($p < 0.05$). The concentrations α-mangostin at 1.25, 2.5, 3.75, 5, 6.25, and 7.5 µM is unable to reduce cell viability of HOC313. **Conclusion:** Low α-mangostin concentrations possibly result in a biphasic effect which leads to increase the viability cell of HOC313 cell line. Therefore, high α-mangostin concentrations might effectively inhibit cancer cell growth, migration, and invasion.

Keywords: Oral squamous cell carcinoma, HOC313 cell, α-Mangostin, Viability test, human and medicine.

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INTRODUCTION

Cancer cell metastasis that spreads from primary tumor tissue to other organs is the main cause of deaths (1). Cancer cells that have undergone epithelial-mesenchymal transition (EMT) can cause reduction and loss of epithelial and interstitial cell adhesion, leading to tumor cell migration and more invasive ability. EMT also deals with therapy resistance against EGFR inhibitor and prevents host immune response to the tumor. TGF–β plays a role as a tumor promotor which can induce EMT (2). OSCC is a cancer cell that has undergone the EMT process. One of the cells is HOC313. OSCC represents around 96% of the oral cavity cancers (3).

Mangostin (*Garcinia mangostana*) contains xanthonoid dan phytochemical (4). Xanthones show antiinflamation, antibacterial, and antioxidant activities (5,6). α-Mangostin is a xanthone derivative that shows anti-cancer activity by stopping cell cycle and apoptosis, inhibiting cell proliferation, and reducing cancer invasion and metastasis. α-mangostin is also proven to inhibit cell viability and morphological changes in OSCC; however, the effective therapeutic concentration as an inhibitor of cell viability is still unclear (7-9).

This study used in vitro culture to treat HOC313 cells with α-mangostin to evaluate the toxicity and viability of the cells. α-mangostin can suppress the invasive ability of EMT and lipopolysaccharide by inhibiting matrix metalloproteinase-2/9 and increasing expression of extracellular-signal-regulated-kinases (ERK)-regulated E-cadherin in pancreatic cancer. Kinases are also proven for its ability to inhibit the viability of cancer cells depending on dose and time of inducing morphological changes in OSCC cell line. (7,10,11)

MATERIALS AND METHODS

This study used an experimental in vitro laboratory study in cell culture of SCC cell line HOC313 which has been

described previously. Viability value was obtained from the results of cytotoxicity test using MTT assay method in HOC313 cell culture treated with α-mangostin at concentrations of 1.25 µM, 2.5 µM, 3.75 µM, 5 µM, 6.25 µM, and 7.5 µM for 24 hours. Data analysis was carried out using the Shapiro-Wilk test and Kruskal Wallis test to identify the relationship between independent and dependent variables.

Sampling criteria

This study used α-mangostin to induce HOC313 cell culture at various concentrations to inhibit cell viability. This study employed an in vitro laboratory experimental design with post-test only control group. α-mangostin is a product of Sigma Aldrich (M3824). In this present study, cells were treated after reaching the confluency level of 80% in 5x103 cells/well. α-mangostin used had six concentrations: 1.25 µM, 2.5 µM, 3.75 µM, 5 µM, 6.25 µM, and 7.5 µM.

Experimental analysis

In this study, the first procedure was thawing HOC313 cells and then performing a cytotoxicity test by treating HOC313 cells with α-mangostin. Procedures for thawing cells were done step by step. First, deliver frozen cells at low temperature to the area of cell culture, and then thawing HOC313 cells from frozen stocks. Afterwards, HOC313 culture was grown into DMEM (37 C; 5% CO2), and then cell culture was passaged to reach 80% confluence. While the cytotoxicity test was employed in some steps, HOC313 cells in suspension (5 x 103 cell/well) were carried out in 96-well plates and incubated for 24 hours (37 C; 5% CO2), and then the medium was replaced. After that, the cells in the well plates were treated with α-mangostin at concentrations of 1.25 µM, 2.5 µM, 3.75 µM, 5 µM, 6.25 µM, and 7.5 µM, and at least 4 samples of replication were made. Treated cells were incubated for 24 hours (37 C; 5% CO2), and 10 µl solution of MTT was added into all the well plates which were then incubated for more 4 hours. After 4-hour incubation, 100 µl SDS–HCL was added to each well, and finally absorbance was measured with a microplate ELISA reader at a 570 nm wavelength, and data analysis was conducted using the Kruskal-Wallis test.

The average Optical Density (OD) could see the percentage of living cells based on the following formula from in vitro technology.

$$\text{cell viability (\% from control)} = \frac{\text{The OD value of treatment group}}{\text{The OD value of control group}} \times 100$$

Data Analysis

Data of the research results was processed in a statistical test.

RESULTS

The data from the cytotoxicity test using the MTT assay method were analyzed in SPSS. The OD measurement was performed using an ELISA reader, and the normality of the data was investigated using the Shapiro Wilk test. From the statistical test, a p-value greater than 0.05 means H0 was accepted. This result indicates the data were normally distributed. After the normality test, the homogeneity of the data was investigated using the Lavene's test. It shows a p-value of 0.011 ($p < 0.05$), meaning that the data were not homogeneous. Following the step, the Kruskal-Wallis test with a significance level of 95% ($p = 0.05$) was employed to identify the normality of the data using the Shapiro-Wilk test.

The average optical density (OD) indicates the number of living cells in sequence. Figure 1 shows the error bar of the research among the different concentration and the control group. In the control group, the average OD at the 1.25 µM concentration of α-mangostin was 0.83. Then, the average OD at the 2.5 µM concentration was 0.8725, while it was 1.008 at the 3.75 µM concentration. This study also obtained the average OD at the 5 µM concentration was 0.991. Furthermore, the average OD at the 6.25 µM concentration was 1.052. Finally, the average OD of 1.008 was observed at the 7.5 µM concentration. While the control group had the average OD of 0.447. There were 28 samples in which consisted of seven sample groups and 4 samples per each group.

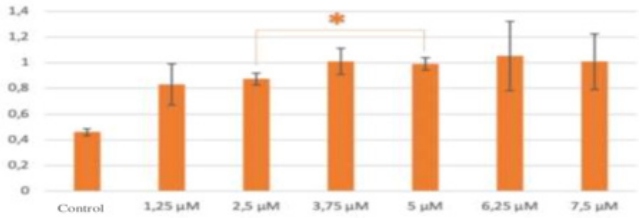

Figure 1: The Average Optical Density (OD) & Standards Intersection of Cells indicates the number of living cells of HOC313 cell line of α -Mangostin.

Based on the Shapiro Wilk test, the significance value was more than 0.05, meaning the data from the seven groups were normally distributed. The test for the homogeneity of variance shows that the variance of the seven groups was different if seen from the p-value $p = 0.011$ ($p < 0.05$). The cytotoxicity test calculated the number of living cancer cells (cell viability). The data from the test were analyzed using the Kruskal-Wallis test with a significance level of 95% ($p = 0.05$) in SPSS. If a p-value is less than the critical limit of the study, H0 is rejected. It means an independent variable affects the dependent variable. From the output above, a p-value obtained was at 0.023 ($p < 0.05$). In other words, concentration of α-mangostin had a significant effect on cell viability. The Mann-Whitney test (Table I) was

Table I: Mann-Whitney test results between control and treatment groups of α-Mangostin

	P1 (1,25 µM)	P2 (2,5 µM)	P3 (3,75 µM)	P4 (5 µM)	P5 (6,25 µM)	P6 (7 µM)
Control	**0.029***	**0.029***	**0.029***	**0.029***	**0.029***	**0.029***
P1 (1,25 µM)		0.773	0.149	0.146	0.149	0.248
P2 (2,5 µM)			0.083	**0.02***	0.149	0.386
P3 (3,75 µM)				0.772	0.773	1.00
P4 (5 µM)					0.772	1.00
P5 (6,25 µM)						0.773

also performed to identify significant differences in cell viability between control cells and all treatment cells. This study found significant differences in cell viability occurred between control cells and all treatments were at 2.5 mg/ml and 5 mg/ml respectively with the value p = 0.02 ($p < 0.05$).

DISCUSSION

α-mangostin has a strong anticancer effect on the oral squamous cell carcinoma (OSCC) through apoptosis and cell cycle arrest. α-mangostin also inhibits cell viability, depending on the dose and time to induce morphological changes in the OSCC (7,12,13). However, the optimal dose of α-mangostin to inhibit the viability of OSCC is unidentifiable. Therefore, this study focused on this issue by investigating different concentrations of α-mangostin, such as 1.25 µM, 2.5 µM, 3.75 µM, 5 µM, 6.25 µM, and 7.5 µM. A previous study by Kwak et al. (7) has shown that α-mangostin at the concentration range of 0-10 µM caused an accumulation of cells in the G1 phase followed by up-regulation of p27kip1 and p21waf1/cip1 known to be CDK inhibitors. Administering α-mangostin to the HSC2, HSC3, HSC4 (cell lines that were established from tumour metastatic lymph nodes originated in oral squamous cell carcinomas) could lower the regulation of the cyclin/CDK complex (cell-cycle control). It means that α-mangostin could inhibit the cell viability in a certain dose and time-dependent manner and induce morphological changes like in epithelial cells.

Oral cancers can progress in four stages and metastasize to distant sites. Metastasis consists of uncontrolled proliferation, angiogenesis stimulation, release of the bloodstream, motility of the bloodstream, invasion of the bloodstream, and the intersection of new microenvironment components, including parenchyma cells, stroma, and inflammation (14-16). EMT is a component that supports tumor invasion and metastasis. During EMT, morphological and genetic changes occur in the primary site of epithelial cells, leading to the growth of mesenchymal to form a secondary tumor elsewhere (5). Previous studies have suggested N-cadherin expression in the HOC-313 cell lines and the loss of E-cadherin expression, and the involvement of EMT. HOC-313 is a cancer cell that undergoes EMT, is highly invasive, and shows spindle morphology without E-cadherin expression (17). In this study, the samples used were HOC313 cells (squamous cell carcinoma) that underwent EMT. A cytotoxicity test was performed using the MTT Assay method. Based on this method, an enzymatic reaction will occur when the tetrazolium salt reacts with living cells, and succinate dehydrogenase yields formazan crystals. The purple formazan formed indicates the presence of living cells. The more intense the formazan's color is, the more alive the cells are (18). The percentage of living cells derived from the cell viability formula based on the optical density (OD) value, treating cells with a 1.25 µM α-mangostin concentration resulted in 147.55% living cells. At the 2.5 µM and 3.75 µM concentrations, there were 153.17% and 170.93% living cells, respectively. As many as 158.23% living cells grew when the 5 µM concentration was given. Furthermore, 176.75% and 171.27% living cells grew at the 6.25 µM and 7.5 µM concentrations.

This study figured out the α-mangostin concentration could increase the viability of HOC-313 cell lines except the concentration range of 3.75 µM to 5 µM. Meanwhile, 6.25 µM and 7.5 µM concentrations could result in a high level of vimentin and low level of E-cadherin in HOC313 cells similarly to HSC2, HSC3, and HSC4 cells. HOC313 cells underwent EMT, while HSC2, HSC3, HSC4 cells showed characteristics of epithelial tissues (19). As a result, HOC313 cells are more invasive squamous cell carcinoma which are relatively insensitive at low α-mangostin concentrations. In another side, Cheng et al. (2) investigated the biphasic effect of cadmium (Cd) on cell proliferation in human fetal lung fibroblast cells. They stated that Cd induced significant cell proliferation at low concentrations but significantly inhibited cell growth at high concentrations. Thuneke et al. (20) also reported a biphasic response of T47D cell proliferation in breast cancer to medroxyprogesterone-acetate (MPA) treatment, which stimulated proliferation and inhibition of T47D cell.

CONCLUSION

As the initial result of this current study, low α-mangostin concentrations possibly result in a biphasic effect on HOC313 cell, which leads to increase the viability cell of HOC313 cell line. Therefore, the potential of α-mangostin at higher concentrations might effectively inhibit cancer cell growth, migration, and invasion. The study recommends further research to find out more about the role of α-mangostin concentration in reducing a number of living cells in Squamous Cell Carsinoma related to biphasic effect.

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