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Cytotoxicity of Philippine Carrageenan in COS-7 Cells

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Abstract

This study is about the cytotoxicity of Philippine carrageenan (*kappa, iota* and *lambda* type) in COS-7 cells (African green monkey cell line producing T antigen of SV40). Results showed that among the carrageenan samples tested, *lambda* carrageenan has the highest cytotoxic activity as observed in the decrease of about 47.6 % cell viability when cells are incubated in longer time (48 h) with 0.0025% concentration. Shorter incubation period did not show any significant cytotoxic effect. Using a three-fold increase in concentration from 0.0025% to 0.0075%, *lambda* carrageenan gave the highest toxicity reading as compared to other types of carrageenan. Mild acid hydrolysis of carrageenan did not show high cytotoxic effect. Changes in cell morphology were observed at 48 hours incubation with *lambda* carrageenan.

Key words : Cellular toxicity, cell viability, cell morphology, cytotoxicity, MTT assay

INTRODUCTION

Cellular toxicity or cytotoxicity is designed to evaluate the acute adverse biological effects of any substances or compounds. It is an important tool in the assessment and evaluation of polymer complex with DNA in gene delivery system. The importance of this study is to investigate the cytotoxic activity of Philippine carrageenan for potential use in gene delivery system. COS-7 cells, derived from CV-1 (a simian cell line *Cercopithecus aethiops*) which can support the growth of SV-40 viruses [1] were used in this study.

Carrageenan are extracts of red seaweeds found in various parts of the world. Extracts from these seaweeds are primarily sulfated polysaccharides of varying ester content[2] (Figure 1) that give the three basic types of carrageenan (*kappa, iota, lambda*) their interesting and unique properties[3]. It is used extensively as a suspending, stabilizing and emulsifying agent in food products [4] as well as pro-inflammatory agent for screening potential anti-inflammatory agents [5]. Although numerous studies were made on the cytotoxicity of carrageenan, these were done mostly in normal human cells like: mammary myoepithelial cells [6]; ileal epithelial cells [7]; vaginal epithelial cells [8]; blood and peritoneal exudate cells [9]; natural killer cells [10]; peripheral blood mononuclear cells [11]; macrophage cytotoxicity [12]; aside from rat liver epithelial cells [13] and bovine brain microvessel endothelial cells [14]. No studies were conducted on cancer cells except in Hela cells [15].

METHODOLOGY

Different samples of Philippine carrageenan were used in this study. These are: *kappa*-type carrageenan (Bengel KK-100); *kappa*-type carrageenan (provided by Marcel Trading Corporation, Quezon City, Philippines); *iota*-type carrageenan (Benvisco SI-100); *lambda*-type carrageenan (Benvisco SL-100); *iota*-type carrageenan (Fluka Chemika-Biochemika, Buchs, Switzerland); *lambda*-type carrageenan (Sigma C-3889, Sigma Chemicals Co. St Louis, USA). Carrageenan with Bengel and Benvisco brands were provided by Shemberg Marketing Corporation, Mandaue City, Cebu, Philippines). Preparation of carrageenan samples (0.0025%, 0075%) were done by dissolving the different samples in phosphate buffer solution (PBS) and heated at 90^{0}C-100 ^{0}C for 5-10 min to completely dissolve the carrageenan. Mild hydrolysis of carrageenan samples at pH 3.0 was also done to test the cytotoxic effect of degraded carrageenan samples. The resulting mixture was then lyophilized and dissolved in PBS solution using the concentration of 0.0025% and 0.0075%.

COS-7 cells were cultured in Dulbecco's modified eagle medium with 10% fetal bovine serum, penicillin and streptomycin at 37^{0}C and 4% CO_2 in a humidified incubator. These were grown to confluent monolayers (3 days) before being used for cell viability. Viability of the cells following exposure to different carrageenan samples was evaluated using the MTT assay (WST-1/1-methoxy-PMS), by the protocol of Dojindo Laboratories, Japan. Confluent COS-7 cells were seeded in quadruplicate with 1.5 x 10^3 cells per cm^2 per well in 96 well plate with their corresponding growth medium as mentioned before. Confluent COS-7 cells were exposed to carrageenan samples at 0.0025% and 0.0075% concentration for 3, 24, 48, 72 h incubated at 37 ^{0}C. After incubation, 10 µL of MTT solution was added followed by incubating again at 37 ^{0} C for 2 h. Absorbance readings were taken at 450 nm using a Lab systems Multiskan MS microplate reader. Cell viability was expressed as a percent viability compared to control untreated COS-7 cells. MTT solution was prepared by mixing WST-1 in PBS, 16.5 mg/4.5 ml, with 1-methoxy PMS, 0.7 mg/ml at a volume ratio of 1:9, (PMS:WST-1).

RESULTS

Figure 2 showed the calibration graph for the MTT assay. The graph showed a straight line wherein cell number was determined using the formula: y = 7043.2x-1103.5. Cell viability was determined based on the blank or control which is untreated. Using a 0.0025% concentration of various carrageenan samples, the MTT % cell viability (Figure 3) was assessed with incubation

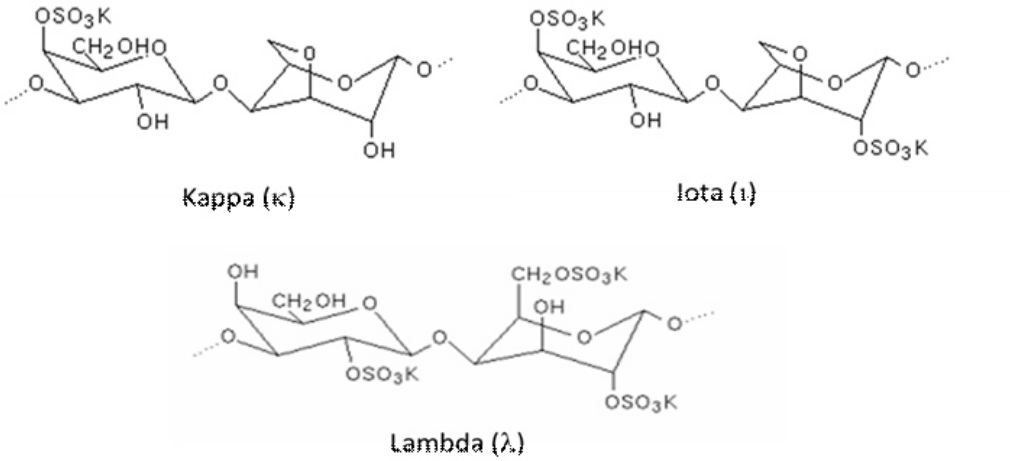

Figure 1: Chemical structures of carrageenan [2]

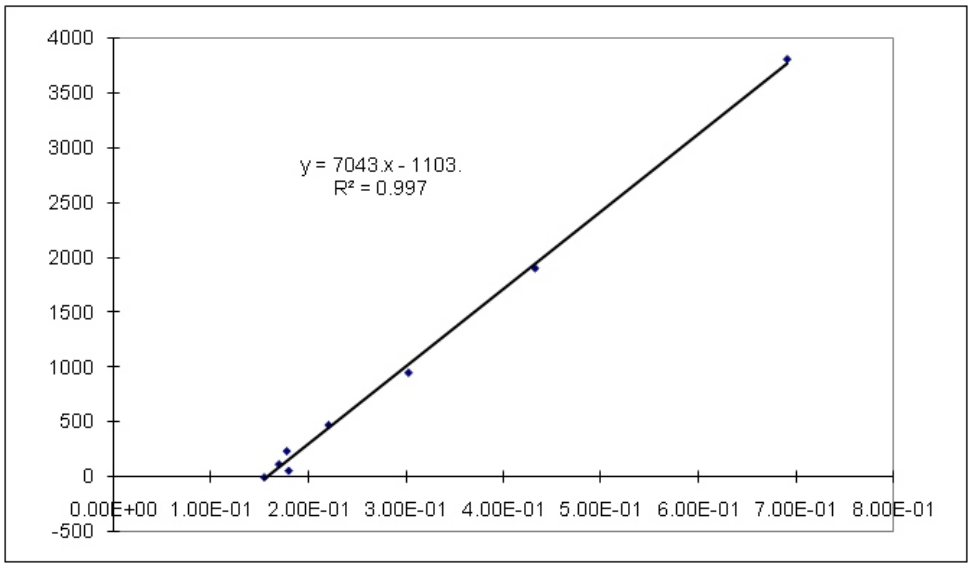

Figure 2: Calibration Graph for MTT Assay

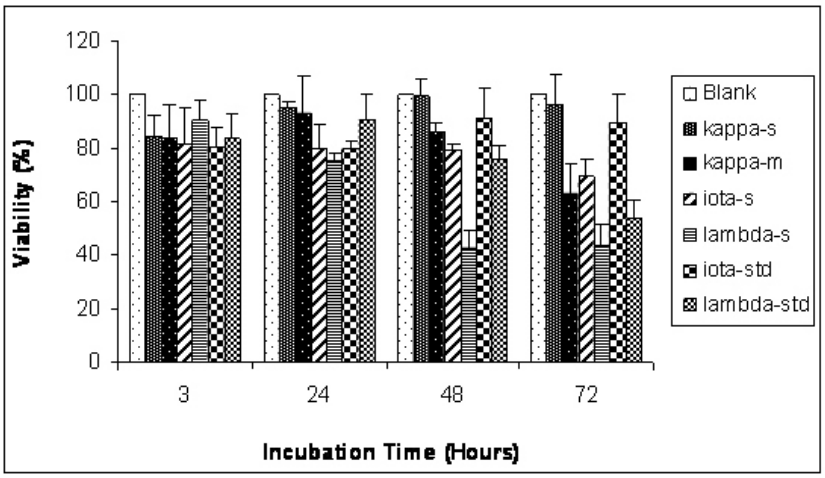

Figure 3: MTT cell viability of COS-7 cells monolayers following incubation in media with 0.0025% concentration of various carrageenan samples.The values represent the mean ± SD, *n* = 4. *s* represents carrageenan samples procured from Shemberg Marketing Corporation, Mandaue City, Cebu, Philippines; *m* represents carrageenan sample procured from Marcel Trading Corporation, Quezon City, Philippines; *iota-std* represents carrageenan sample procured from Fluka Chemika-Biochemika, Buchs, Switzerland; *lambda*-std carrageenan sample procured from Sigma Chemicals Co. St Louis, USA.

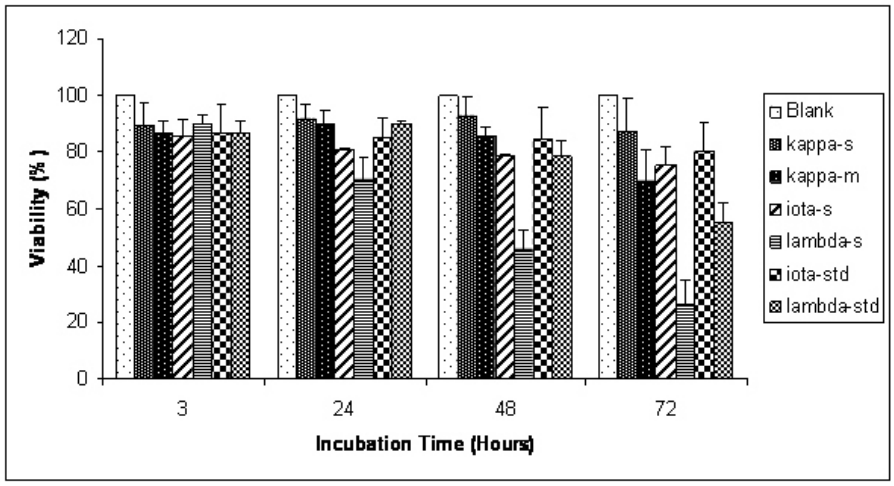

Figure 4: MTT cell viability of COS-7 cells monolayers following incubation in media with 0.0075% concentration of various carrageenan samples.The values represent the mean ± SD, *n* = 4. *s* represents carrageenan samples procured from Shemberg Marketing Corporation, Mandaue City, Cebu, Philippines; *m* represents carrageenan sample procured from Marcel Trading Corporation, Quezon City, Philippines; *iota-std* represents carrageenan sample procured from Fluka Chemika-Biochemika, Buchs, Switzerland; *lambda-std* carrageenan sample procured from Sigma Chemicals Co. St Louis, USA.

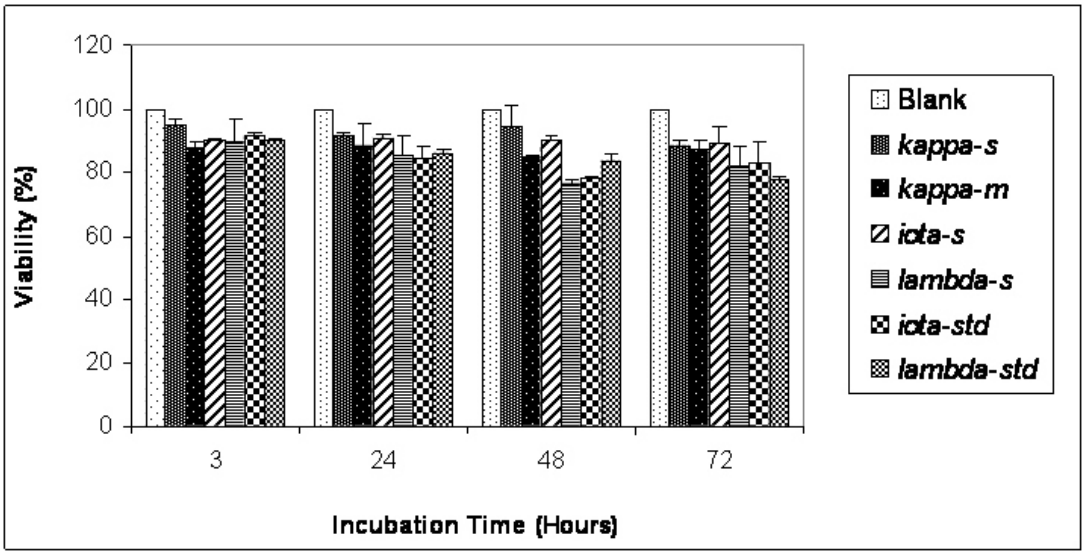

Figure 5: MTT cell viability of COS-7 cells monolayers following incubation in media with 0.0025% concentration of various hydrolyzed carrageenan samples. The values represent the mean ± SD, *n* = 4. *s* represents carrageenan samples procured from Shemberg Marketing Corporation, Mandaue City, Cebu, Philippines; *m* represents carrageenan sample procured from Marcel Trading Corporation, Quezon City, Philippines; *iota-std* represents carrageenan sample procured from Fluka Chemika-Biochemika, Buchs, Switzerland; *lambda-std* carrageenan sample procured from Sigma Chemicals Co. St Louis, USA.

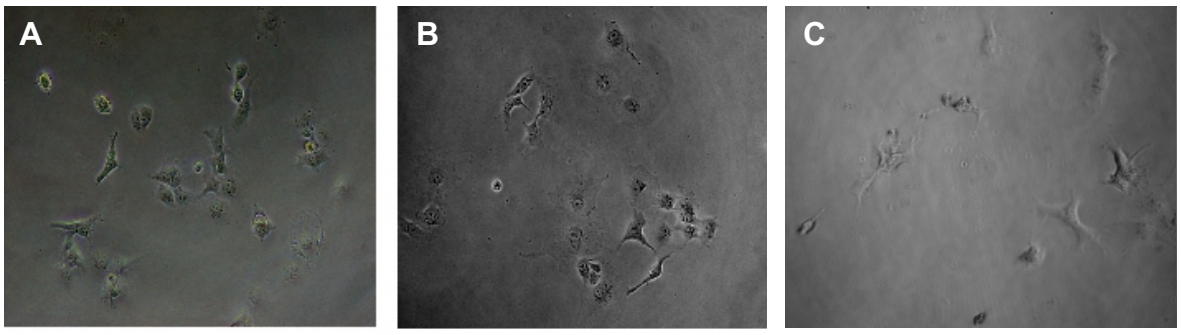

Figure 6: Images of COS-7 cells. A, untreated COS-7 cells (control) B, COS-7 cells treated with 0.0025% *lambda-s* carrageenan incubated for 48 h C, COS-7 cells treated with 0.0075% *lambda-s* carrageenan incubated for 48 h.

time of 3, 48, 72 h. The graph showed that among the carrageenan samples tested, *lambda* carrageenan has the highest toxicity level as observed in the decrease of about 47.6 % cell viability when cells were incubated for 48 h. Same behaviour (reduction of cell viability) was also observed in *lambda* carrageenan procured from Sigma Chemicals which is being used as standard. Shorter incubation period did not show any significant cytotoxic effect. Significant cytotoxic effects of carrageenan were observed at 48 hours incubation. *Kappa* carrageenan has the least cytotoxic effect.

Increasing the concentration of carrageenan samples by three fold, also showed a much higher cytotoxic effect. The cell viability compared to control with incubation period of 3, 24, 48, 72 h is shown in Figure 4. Again *lambda* carrageenan exhibited a higher cytotoxic effect as compared to other types of carrageenan.

The results of MTT cell viability of hydrolyzed carrageenan using a 0.0025% concentration is shown in Figure 5. Mild hydrolysis of carrageenan did not show a significant cytotoxic effect in COS-7 cells.

Photographs of untreated COS-7 cells (control), and treated COS-7 cells with *lambda* carrageenan (0.0025% & 0.0075%) exposed for 48 h is shown in Figure 6. Some changes in the cell morphology were observed in the treated cell with *lambda* carrageenan as compared to the control.

DISCUSSION

In this study, various types of carrageenan were investigated for its cytotoxic effects in COS-7 cells. Using in vitro technique, cell viability was determined. MTT cytotoxicity assay was performed to determine the viability of COS-7 cells exposed to different types of carrageenan at concentration ranging from 0.0025% to 0.0075%. Results indicate that only *lambda* carrageenan affected the COS-7 cell viability (Figures 3, 4 & 5). Longer exposure time with *lambda* carrageenan greatly affected the cell viability. However, between the two *lambda* carrageenan samples, the sample provided by Shemberg Marketing Corporation, Philippines has the highest cytotoxic effect than the sample bought from Sigma Chemical Co., USA. The same results was also observed in the study conducted by Huber et al [14], of *lambda* carrageenan procured from Sigma (St. Louis, MO). It did not show significant decrease in cell viability when used in endothelial cell viability assay. At a concentration of 0.0015% to 0.015%, *lambda* carrageenan did not affect the permeability coefficient of bovine brain microvessel endothelial cell monolayers which is directly related to the decreased cell viability. Therefore the use of higher concentration of 0.0025% to 0.0075%, *lambda* carrageenan exhibited a cytotoxic effect in COS-7 cells. According to Suzuki et al [13], *lambda* carrageenan was not cytotoxic at 0.001% after three days treatment in normal rat liver epithelial cell line WB-F344. At 0.01% and 0.1% concentration showed slight cytotoxicity, although most of the surviving cells appeared as normal as the non-treated cells under a microscope. The result of the cell viability assay using hydrolyzed carrageenan at 0.0025% concentration is shown in Figure 5. Mild hydrolysis of carrageenan did not show high cytotoxic effect in COS-7 cells. This might be attributed to the higher concentration of salts accumulated in the hydrolysis since the samples were not purified right after the hydrolysis. Instead the samples were just lyophilized prior to dissolution in phosphate buffer solution. Figure 6 showed the cell morphology of control and sample treated with *lambda* type carrageenan incubated for 48 h. Some changes in the cell morphology were observed in the treated cell with *lambda* carrageenan. Treated cells were of smaller size than the untreated cells. This might due to the cytotoxic effect of *lambda* carrageenan in the cells. Based on the cellular cytotoxicity analysis of carrageenan samples, *kappa* and *iota* types are the potential candidates for gene/drug delivery system.

CONCLUSION

The results of the cytotoxicity analysis of Philippine carrageenan (*kappa, iota* and *lambda*) in COS-7 cells showed that *kappa* and *iota* types can be safely use in gene/drug delivery system based on their low cytotoxic effects. *Lambda* type had higher cytotoxic activity as observed in the decrease of cell viability and change in cell morphology when cells are incubated in 48 hours. Shorter incubation period did not show any significant cytotoxic effect.Mild acid hydrolysis of carrageenan did not show high cytotoxic effect.

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