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The Effects of Zeolite X and Y on Cancer Cell Lines

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Abstract

Zeolites are hydrated silicates of aluminium that have been very useful in many industry because of its microporous property, absorbance ability and ion exchange capacity. It is currently viewed as a potential adjuvant in cancer therapy due to its ability to inhibit the proliferation of cancer cells. Research on natural zeolite clinoptilolite application as anticancer agent has been proven by others. However, the effect of other types of zeolite on cancer cells is still uncertain. This study is performed to determine the effects of zeolite X and Y on cancer cell lines proliferation *in vitro*. Cancer cell lines HeLa, AsPC-1 and 911 cells were cultured in designated medium treated with zeolite X and zeolite Y at the concentration of 5 mg/ml and 50 mg/ml. Fetal Bovine Serum (FBS) concentrations were modified to 5%, 10%, 15% and 20%. After 72 hours incubation, the efficacy of zeolite to treat cancer cell lines were measured by means of cell viability test via MTT assay. Overall results showed that cancer cell lines cultivated in the medium treated with 50 mg/ml of zeolite X and 5% FBS exhibited the highest inhibition of cell proliferation and decrease in cell viability. This finding provides preliminary information in the study of determining the potential use of zeolite as anticancer agent for alternative or complementary therapy.

Keywords: Zeolite X; zeolite Y; anticancer agent; cancer therapy; complementary therapy

1. INTRODUCTION

Cancer is an uncontrolled growth and spread of abnormal cells caused by several intrinsic and extrinsic factors such as inherited mutations, hormones, immune conditions, chemicals, radiations and infectious agents. It is one of the major diseases in the world that are linked to a high death toll. According to the latest statistics in 2008 from the International Agency for Research on Cancer (IARC), the two most common types of new cancer cases and deaths among South East Asian men are lung cancer (19.8%) and liver cancer (15.1%) while breast cancer (22.4%) and cervical cancer (11.4%) remain as the top two cancer types in South East Asian women [1].

Cancer is usually treated by surgery, radiation therapy, chemotherapy and hormone therapy. Besides that, there are a few other existing options in cancer treatments that are still under research or yet to be proven clinically, for example gene therapy and other complementary therapies such as the use of plants, fruits or herbal extracts. Unfortunately, there are still many limitations to the current varieties of cancer treatment. One of the main constraint is side effects, especially after a chemotherapy. Swelling, fatigue, nausea, vomiting and headache are the usual complaints acknowledged among cancer patients. The possibility of recurrence and the grief of losing an organ after a surgery are some added constraints in the present cancer therapies.

Zeolites are hydrated aluminium silicates that are widely used in many industries and agriculture. Additionally, zeolites have been useful in biomedical application such as in the treatment of diabetes mellitus, antidiarrheal agents, hemodialysis, bone formation and drug delivery [2]. Earlier in the last decade, tribomechanically micronized zeolite was revealed to have anticancer and antioxidative effect on several human cell lines [2]-[3]. Natural clinoptilolite is viewed as a potential adjuvant in cancer treatment due to its ability to inhibit the proliferation of cancer cells [2], [4]. However, the effect of other types of zeolite on cancer cells is still uncertain. This study is performed to determine the effects of zeolite X and Y on cancer cell lines proliferation *in vitro*.

2. MATERIALS AND METHODS

Complete growth medium of Dulbecco's modified Eagle's medium (DMEM) and Roswell Park Memorial Institute medium (RPMI 1640) were used for cell culture. MTT solution (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide, a yellow tetrazole) prepared using MTT powder by Sigma-Aldrich was used for cell viability assay. MTT solvent was prepared using hydrochloric acid and isopropanol.

2.1 Cell Culture

Three cancer cell lines were used in this study; cervical carcinoma (HeLa), human pancreatic tumor (AsPC-1) and human embryonic retinoblasts (HER) cells (911), which were obtained from American Type Culture Collection (ATCC) Biological Resource Centre. The cells were grown in tissue culture flasks containing media DMEM or RPMI 1640, supplemented with different concentrations (5%, 10%, 15%, 20%) of fetal bovine serum (FBS), 2% L-glutamine, 1% penicillin-streptomycin in a humidified atmosphere with 5% CO_2 at 37°C. Then, the cells were isolated and 200 µl of the cell suspension were seeded into 96-well plates ($1x10^4$ cells/ml) using the appropriate medium in triplicates. Upon reaching 60% to 80% confluency, cell medium in the 96-well plates was removed and replaced with medium which was pre-treated with 5 mg/ml or 50 mg/ml zeolite (zeolite X, zeolite Y). Cells were then incubated for 24 to 72 hours in a CO_2 incubator.

2.2 Zeolite Treatment of the Media

Zeolites X and Y were weighed and added into each represented serum-free media at 5 mg/ml and 50 mg/ml. The solution was autoclaved for 20 minutes at 121°C, liquid cycle. After that, the solution was centrifuged at 26, 000 rpm for 5 minutes. The solution was then separated from its suspension. Finally, different concentrations (5%, 10%, 15%, 20%) of fetal bovine serum (FBS), 2% L-glutamine and 1% penicillin-streptomycin were supplemented into the treated-media.

2.3 Cell Viability Assay

After 72 hours of incubation, the treated-medium was removed and 90 µl complete growth medium was added in each 96-well together with 10 µl of MTT solution per well. The cells were then incubated for 4 hours in CO_2 incubator at 37°C in dark. Later, MTT solvent was added and the results were read by ELISA micro-well plate reader at 575 nm.

3. RESULTS AND DISCUSSION

The data collected from this study were analysed accordingly using ANOVA Tukey's test. The analysis were performed between the data of the control and the data from the different FBS concentrations. Significant differences were shown when $p \leq 0.05$.

3.1 Treatment of HeLa with Zeolite X and Y

Zeolite X showed a higher cell growth inhibition than zeolite Y at 5 mg/ml zeolite with 15% and 20% FBS (Fig. 1a). On the other hand, zeolite Y showed a higher inhibition than zeolite X at 5 mg/ml zeolite with 5% FBS and 10% FBS. Statistically, treatment using zeolite X at 5 mg/ml showed no significant difference between the control and any of the FBS concentrations used. A significant difference can only be seen when HeLa was treated using 5 mg/ml of zeolite Y at 15% FBS in compare to other FBS concentrations.

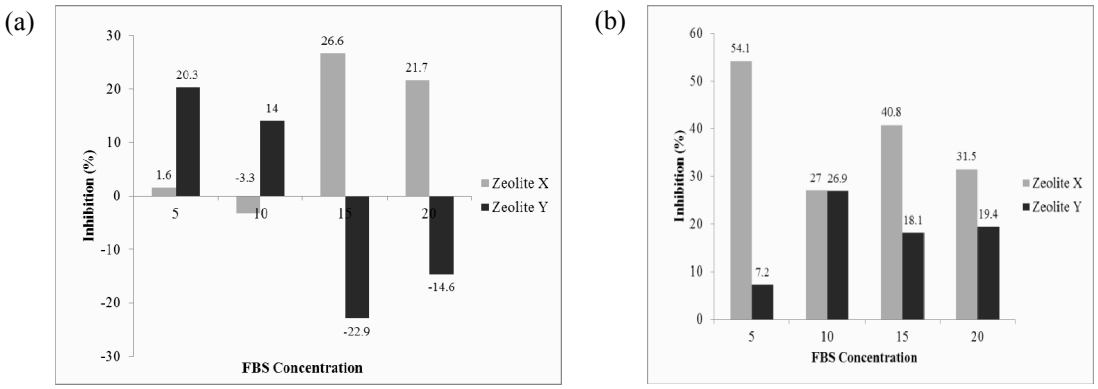

Figure 1: Inhibition of HeLa using Zeolite X with (a)Y (5 mg/ml) and (b) Y (50 mg/ml).

Figure 1b shows the inhibition of HeLa when treated with zeolite X and Y at concentration 50 mg/ml. Zeolite X showed a higher cell growth inhibition compared to zeolite Y. Treatment of medium using 50 mg/ml of zeolite X showed a statistically significant difference at 5% FBS. However, there was no significant difference shown between the control and any of the FBS concentrations in the medium treated with 50 mg/ml of zeolite Y.

3.2 Treatment of 911 with Zeolite X and Y

In general, human embryonic retinoblasts (HER) cells known as 911, showed a higher decrease in viability after treatment with zeolite X at 5 mg/ml compared to after treatment with zeolite Y at 5 mg/ml (Fig. 2a). Statistically significant difference between different FBS concentrations were shown in cell cultures with zeolite X treatment. However, treatment using zeolite Y at 5 mg/ml showed significant differences between the control and all the different concentrations of FBS but no significant differences were observed within the different FBS concentrations.

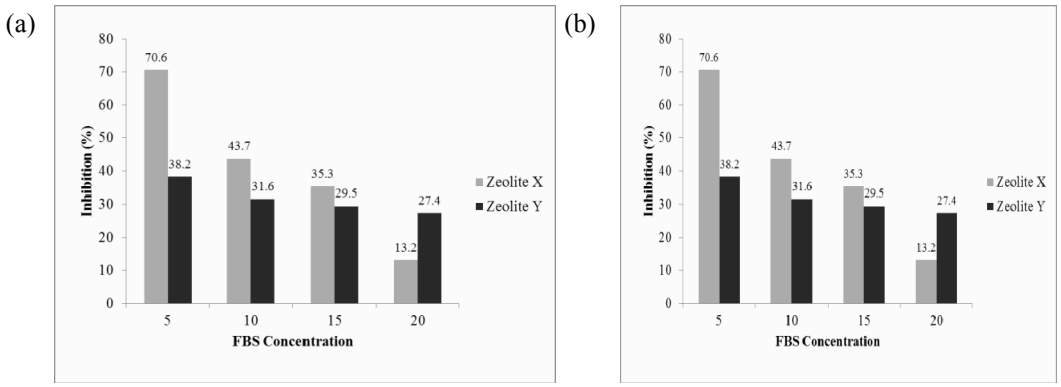

Figure 2: Inhibition of 911 using Zeolite X with (a) Y (5 mg/ml) and (b)Y (50 mg/ml).

Zeolite X showed a higher decrease in 911 cell viability compared to zeolite Y at concentration 50 mg/ml (Fig. 2b). Treatment using zeolite X at 50 mg/ml showed significant differences between the control and all the different FBS concentrations but there were no significant differences among the different concentrations. On the other hand, a significant difference can be seen when 911 was treated using 50 mg/ml zeolite Y at all different FBS concentrations.

3.3 Treatment of AsPC-1 with Zeolite X and Y

Generally, zeolite X treatment showed a higher inhibition compared to zeolite Y treatment. Figure 3a shows the inhibition of AsPC-1 when treated with zeolite X and Y at 5 mg/ml concentration. Statistic analysis showed that the control results from both treatments were significantly different from the results of all their FBS concentrations. Zeolite Y treatment exhibited a higher inhibition of cell proliferation compared to zeolite X treatment at 50 mg/ml concentration (Fig. 3b). Statistic results for both zeolites treatment showed significant differences in all FBS concentrations.

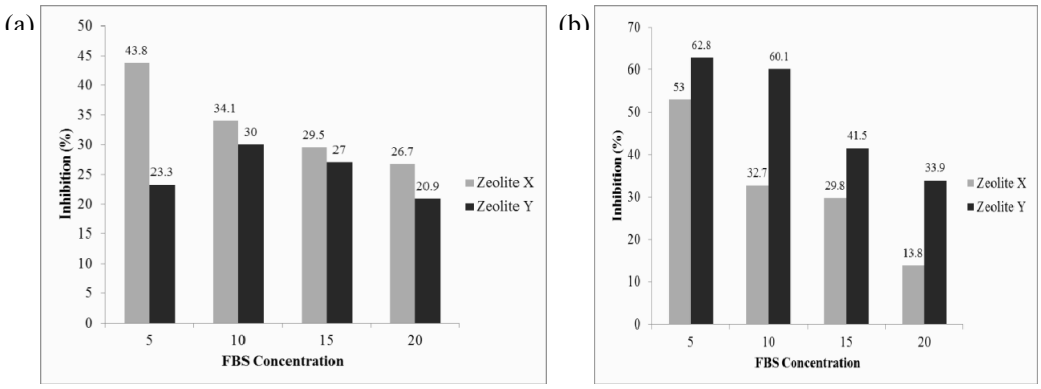

Figure 3: Inhibition of AsPC-1 using Zeolite X with (a) Y (5 mg/ml) and (b) Y (50 mg/ml).

3.4 Paired sample T-test

In this study, the T-test was used to compare between; (i) zeolite X and Y at 5 mg/ml, and (ii) zeolite X and Y at 50 mg/ml. By calculation, only HeLa showed $p < 0.05$, indicating that zeolite X and Y showed a significant difference at 5 mg/ml (Table 1).

Table 1: Paired sample T-test between zeolite X and Y at 5 mg/ml.

	Cell Lines	Sig. (p)
Zeolite X-Y (5 mg/ml)	HeLa	0.001
	911	0.087
	AsPC-1	0.783

Nonetheless, all three cell lines at 50 mg/ml showed $p < 0.05$, which indicates that zeolite X and Y showed a significant difference for all tested cells (Table 2).

Table 2: Paired sample T-test between zeolite X and Y at 50 mg/ml.

	Cell Lines	Sig. (p)
Zeolite X-Y (50 mg/ml)	HeLa	0.006
	911	0.000
	AsPC-1	0.028

Zeolite X was found to be a higher inhibitor than zeolite Y according to results obtained in this study. The reason could be because zeolite X has a higher adsorption property compared to zeolite Y. Commercial zeolite X from Zeolyst has a larger surface area of 925 m^2/g compared to nanocrystalline of Zeolite Y, which is only 648 m^2/g as measured by [5]. Hence, the higher adsorption property of zeolite X than zeolite Y is equitable.

4. CONCLUSIONS

Briefly, zeolite X and Y inhibited cancer cell lines proliferation and decrease the cells viability *in vitro*. Overall results showed that cancer cell lines cultivated in the medium treated with 50 mg/ml of zeolite X and 5% FBS exhibited the highest inhibition of cell proliferation and decrease in cell viability. The best cell medium for treatment of cancer cell lines using zeolites are the ones with low FBS concentration. The different results obtained when FBS concentration were manipulated proved that added serum in cell media influenced zeolite efficacy as anticancer adjuvant. The findings provide preliminary information in the study of determining the potential use of zeolite as anticancer agent for alternative or complementary therapy.

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