

Original Article

A Novel Assessment of Sugar in Oral Squamous Cell Carcinoma Treated with Cisplatin

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Abstract

The Background: Cancer treatment is one of the global challenges in the world. The type and amount of certain dietary components such as sugar could affect response to chemotherapeutic treatment. This research was performed to detect the influence of table sugar, brown sugar and sugar substitute (xylitol) with three different concentrations on the chemosensitivity of squamous cell carcinoma treated with cisplatin chemotherapy. Material & Methods: Dulbecco's modified Eagle medium supplemented with 10% foetal bovine serum was used to cultivate the squamous carcinoma cell line. Cisplatin chemotherapy was added according to the manufactory's guide to culture media. The squamous cell carcinoma cell line was subjected to table sugar, brown sugar, and sugar substitute (xylitol) at 10%, 30%, and 75% concentrations. Cytotoxicity of the cancer cells was detected by MTT assay, in addition cell death (apoptosis) was measured by flow cytometry. Results: Statistical analysis of the collected data revealed that increasing the amount of any type of the three kinds of sugar lead to a significant decrease of chemosensitivity of squamous cell carcinoma (P-value<0.0001). Moreover, the table sugar resulted in a significant increase in chemoresistance of cancer cells concerning cell viability and cell death when compared with brown sugar and sugar substitute(p-value≤0.05). Conclusion: It has been concluded that the worst type of sugar is table sugar followed by brown sugar that increases cancer cell resistance during chemotherapy treatment. However, xylitol (sugar substitute) could be considered as the best type of sugar that can be used without marked affection on the chemosensitivity of cancer cells.

Keywords: Oral cancer; Sugar; cisplatin; chemosensitivity; SCC-25

I. INTRODUCTION

"Let your medicine be your food and your food be your medicine" this principle is very important for cancer patients especially during treatment as their dietary intake can critically be a matter of life and death. Sugar is one of the most harmful dietary components for a cancer patient as it promotes cancer development and metastasis [1].

Several types of research documented that the elevation of the blood glucose during chemotherapy resulting in decreasing the chemosensitivity of the cancer cell [2–5]. In

addition, it was found that the antiproliferative effect of chemotherapy such as 5-Fluorouracil (5-FU) on colon cancer was attenuated by elevation of blood glucose, therefore the patients need higher doses of chemotherapy and longer treatment time [2].

Moreover, Zhao et al. [3] showed that the chemotherapeutic sensitivity of gastric carcinoma to 5-FU was decreased with hyperglycemia. Also, it had been documented that increasing glucose levels may enhance prostate cancer progression by boosting cancer cell proliferation and retarding cancer cell death [4,5].

Sugar is made by refining sugar beets and sugar cane, followed by a purifying procedure that removes molasses, a brown liquid. One of the worst sugars is sucrose, which is found in white sugar (table sugar). It is made up of two sugars: fructose and glucose [6]. Brown sugar can be manufactured by molasses-coating white granulated sugar. It contains approximately 0.25 fewer calories per gram than white sugar. With its modest bit of syrup, it has a slightly less pronounced sweetness [7]. Xylitol (sugar replacement) is a colorless or white crystalline substance that is water soluble. It is a polyalcohol and a sugar alcohol, more specifically an alditol. Because it is processed independently of insulin; xylitol has no effect on blood sugar [8].

Cisplatin is one of the commonly used chemotherapeutic agents that resulting in the death of cancer cells by damaging the DNA [9,10,11]. This study aimed to explain the influence of three kinds of dietary sugar on the therapeutic response and chemosensitivity of squamous carcinoma cell lines treated by cisplatin.

II. MATERIALS AND METHODS

A. Culture of cells

The SCC-25 squamous cell carcinoma cell line was purchased from (ATCC, USA). It was kept in Dulbecco's modified Eagle's medium (DMEM) supplemented with 10% foetal bovine serum (HyClone; GE Healthcare Life Sciences, Logan, UT, USA) (FBS; HyClone; GE Healthcare Life Sciences, Logan, UT, USA). Cisplatin was purchased from Sigma Chemical Co. (St. Louis, MO, USA). The cells were divided into 96-well plates and kept at 37°C in a 5% CO₂ atmosphere for 24 hours. Cells were classified into four groups:

- Group 1: SCC-25 treated with Cisplatin(100µM) alone.
- Group 2: SCC-25 treated with Cisplatin (100µM) and table sugar (sucrose which is made up of glucose and fructose) in 3 different concentrations, 10% & 30% and 75%

- Group 3: SCC-25 treated with Cisplatin (100µM) and Xylitol (sugar alcohol and used as sugar substitution) in 3 different concentrations, 10% & 30% and 75%
- Group 4: SCC-25 treated with Cisplatin(100µM) and brown sugar (table sugar with varying amount of molasses) solution in 3 different concentrations, 10% & 30% and 75%.
- Control group: SCC-25 not treated with Cisplatin.

B. Cytotoxicity detection using the MTT technique

Each batch of cells was treated with MTT (20 l; 5 mg/ml dissolved in PBS; Glenview; SigmaAldrich; EMD Millipore, Billerica, MA, USA) and kept at 37°C for 4 hours. Spectrophotometrically (BioTekElx 800; BioTek Instruments, Inc., Winooski, VT, USA) measure absorbance at a wavelength of 570 nm. Measure the background absorbance of multiwell plates at 690 nm and subtract from the 450 nm measurement.

C. FACSCalibur flow cytometry analysis (BD Biosciences, Franklin Lakes, NJ, USA)

Flow cytometry was performed using an excitation wavelength of 488 nm and an emission wavelength of 53015 nm. The FACSCalibur was used to stain the cells of each group with propidium iodide. An apoptosis detection kit (Annexin V PE/7 AAD) was used to detect apoptosis.

D. Statistical analysis:

The findings were analyzed using IBM Statistical Package for Social Sciences (SPSS), version 21 (SPSS Inc., Chicago, IL, USA). Mean and standard deviation were used to characterize numerical data, and ANOVA and a post-hoc Tukey test were employed to compare groups. P-values were deemed significant when equal to or less than 0.05.

III. RESULTS

We examined the effect of three distinct types of sugar at varying doses on the SCC-25 cell line treated with cisplatin in this

research. The MTT test was used to determine cell cytotoxicity first, and subsequently flow cytometry was utilized to determine cell death.

A. MTT assay

The control group was composed of cancer cells without receiving any treatment (98.35 ± 0.67). In all groups, viable cancer cell number was increased significantly when the concentrations of the sugar increased when compared with group 1 (2.775 ± 0.15). The number of viable cells was significantly increased at 75% concentration in group 2 (table sugar) (11.582 ± 0.66) and group 4 (brown sugar) (6.050 ± 0.12) respectively. The least viable cancer cells were observed in group 3 with xylitol (4.888 ± 0.27) (p-value < 0.0001).

This result indicates that there is a strong relationship between the type and concentration of sugar and the growth of tumor cells. (Table 1, Figure 1).

B. Flow Cytometry

The cell apoptosis and necrosis were decreased significantly in all groups when different types of sugar were added at different concentrations compared to group 1 (34.13 ± 0.8 , 2.99 ± 0.15) respectively. A Significant decrease of apoptosis and necrosis respectively had been observed in group 2 (table sugar) (16.14 ± 0.18 , 3.12 ± 0.50) followed by group 4 (brown sugar) (22.44 ± 0.34 , 2.97 ± 0.29) then group 3 (xylitol) (27.60 ± 0.43 , 3.97 ± 0.13) (p-value < 0.0001). (Table 2, Figure 2 and 3)

Table (1): Cytotoxicity of SCC-25 cell line treated with cisplatin after addition of different types of sugar at different concentrations.

Conc Groups	10%	30%	75%	P-value
Group 2	5.091 ± 0.29^b	6.073 ± 0.34^c	11.582 ± 0.66^d	0.0000
Group 3	3.578 ± 0.2^a	4.367 ± 0.24^b	4.888 ± 0.27^c	0.0001
Group 4	4.715 ± 0.09^a	5.010 ± 0.07^a	6.050 ± 0.12^b	0.0000
P-value	0.000	0.0000	0.0000	

Groups with different letters are statistically significantly different

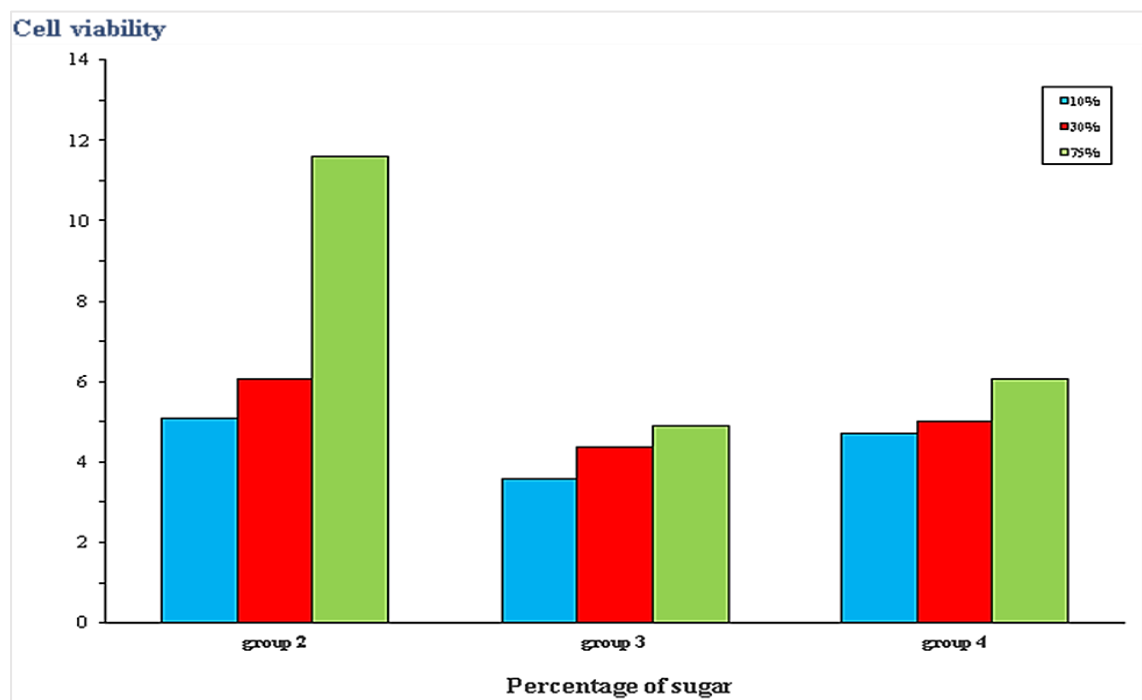


Figure (1): Effect of different types of sugar at different concentrations on viability of SCC-25 treated with cisplatin

Table (2): Apoptosis and necrosis of SCC-25 cell line treated with cisplatin after the addition of different types of sugar at various concentrations.

s		Apoptosis			Necrosis
		Total	Early	Late	
	Control	2.25±0.20 ^a	1.41	0.44	0.42±0.08 ^a
1	Group 1	34.13±0.8 ^a	6.59	25.11	2.99±0.15 ^b
2	Group 2-10%	24.42±0.42 ^a	8.32	12.47	3.46±0.36 ^c
3	Group 2-30%	19.52±0.40 ^b	5.42	11.24	2.90±0.32 ^d
4	Group 2-75%	16.14±0.18 ^c	7.39	5.83	3.12±0.50 ^d
	P-value	0.0000			0.0000
5	Group 3-10%	31.25±0.89 ^b	7.88	21.17	2.74±0.20 ^a
6	Group 3-30%	29.15±0.84 ^c	11.24	15.15	3.02±0.08 ^b
7	Group 3-75%	27.60±0.43 ^d	8.31	15.38	3.97±0.13 ^c
	P-value	0.0000			0.0000
8	Group 4-10%	28.02±0.35 ^a	6.89	18.69	2.79±0.21 ^a
9	Group 4-30%	27.25±0.20 ^a	8.73	15.42	3.12±0.13 ^b
10	Group 4-75%	22.44±0.34 ^b	13.16	6.69	2.97±0.29 ^b
	P-value	0.0000			0.0000

Groups with different letters are statistically significantly different

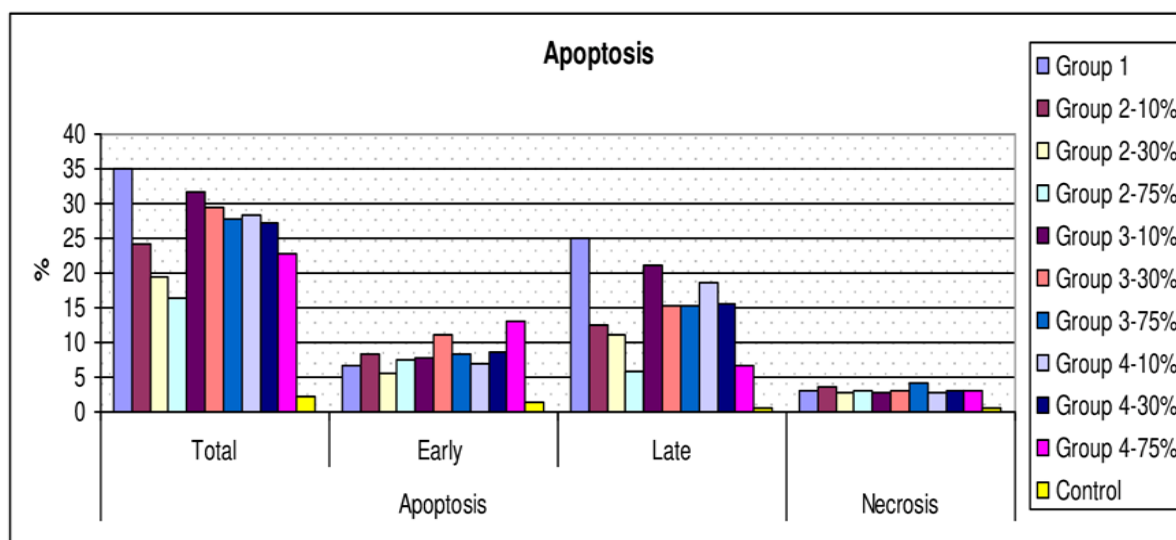


Figure (2): The effect of various sugars at varying concentrations on the apoptosis and necrosis of SCC-25 cells treated with cisplatin

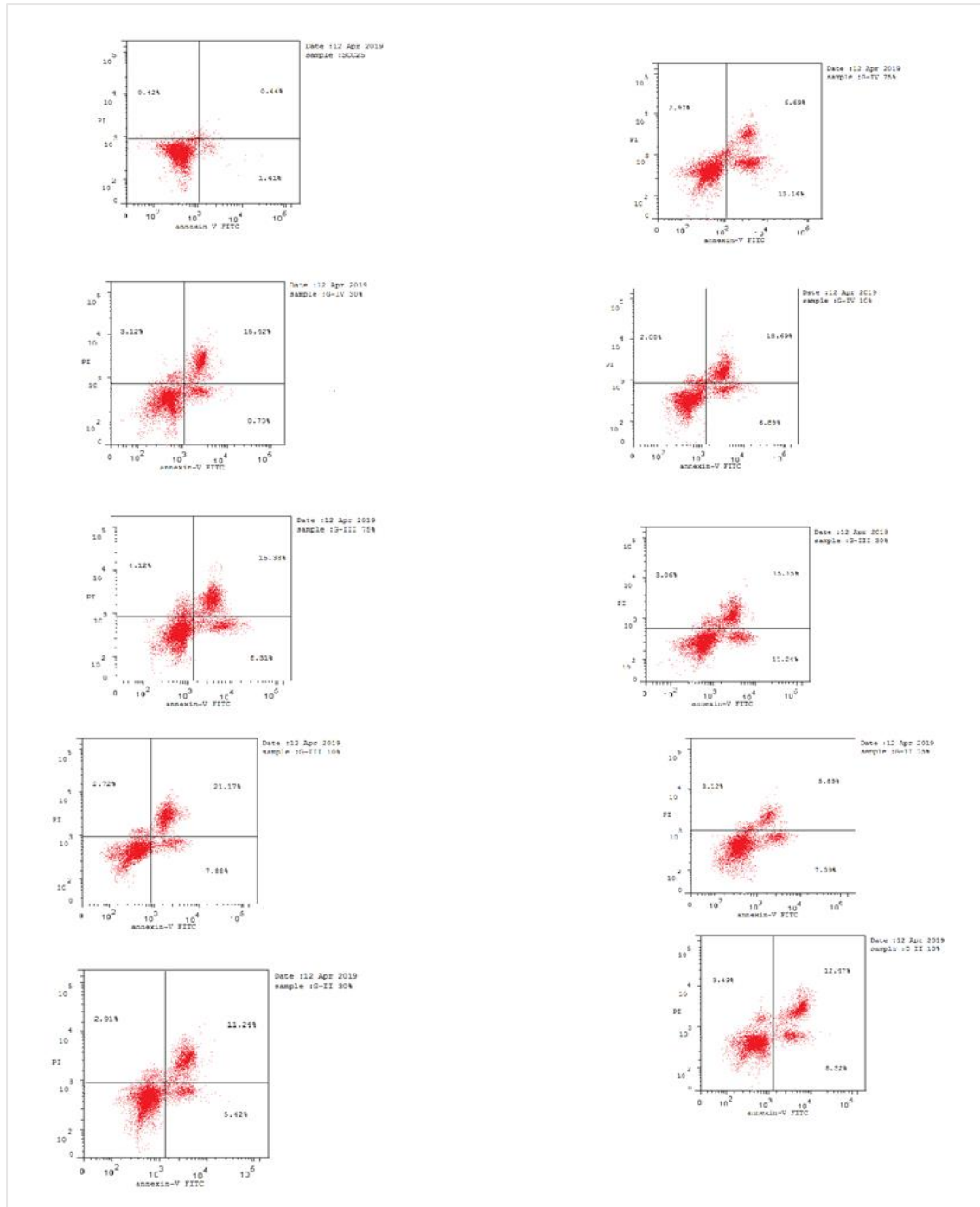


Figure (3): Apoptosis detection using flow cytometry

IV. Discussion

The purpose of this research was to determine the relationship between different types of sugar at different concentrations and chemosensitivity in squamous cell carcinoma treated with cisplatin. It was found that the increase in the concentration of any type of sugar resulted in a significant decrease in

chemosensitivity of cancer cells (p-value=0.000). In addition, it was found that table sugar is the worst type causing a significant increase in the chemoresistance of cancer cells when compared with the other types. However, xylitol is the best type of sugar concerning chemosensitivity.

Our results could be explained by the “Warburg” effect in which the tumor cell performs glycolysis to change glucose to lactic

acid to produce energy in an anaerobic or anoxic environment. Glycolysis results in the decrease of adenosine triphosphate (ATP) therefore, the tumor cells increase the glucose intake to enhance energy-providing glycolysis [1].

Therefore, increasing the concentration of any type of sugar resulted in the enhancement of tumor cell proliferation and resists cancer cell death. The type of sugar which contains a large amount of glucose such as table sugar resulted in the induction of tumor cell proliferation, invasion and migration, and decreasing cell death. However, brown sugar contains glucose less than table sugar, so it leads to less chemoresistance of cancer cells when compared with table sugar. Sugar substitute (xylitol) contain sugar alcohol; therefore, it is the safest type of sugar that can be used by cancer patients it promotes chemosensitivity of cancer cells regarding cell vitality and cell death.

In addition, the results of the present study were confirmed by several studies which found that increasing blood glucose levels during chemotherapy resulted in decreasing chemosensitivity of the cancer cells. Zhao et al. [3] discovered that raising blood glucose levels boosts mutant p53 expression in cancer cells. Wang et al. [12] discovered that p53 mutations were strongly linked to decreased efficacy of platinum-based induction chemotherapy in head and neck squamous cell cancer. As well as it had been stated that increasing blood glucose level promote expression of Nampt, Sirt1 resulting in increasing P-glycoprotein (P-gp) which is a chemoresistance protein, and a decrease of Topoisomerase II α (Topo-II α) which is an anticancer drug target [3].

Moreover, Biernacka et al. [13] pointed out that increasing blood sugar levels promotes the expression of IGFBP2 resulting in resistance of prostate cancer cells to apoptosis that is induced by chemotherapy. It had been documented that IGFBP2 inhibits the phosphatase and tensin homolog (PTEN) which is a tumor suppressor gene, resulting in chemoresistance [14]. Therefore, the IGFBP-2

expression level is correlated positively with the breast, colon, lung, and prostate cancer progression [15].

Additionally, Zeng et al. [16] demonstrated that the chemosensitivity of breast cancer cells in a hyperglycemic environment was connected to fatty acid synthase (FAS). Thus, blocking fatty acid synthase increases chemosensitivity and promotes breast cancer cell death.

Finally, table sugar is the worst type of sugar resulting in chemoresistance of the cancer cells. The sugar substitute (xylitol) could be the safest type of sugar for cancer chemotherapy when compared to other types of sugar. Therefore, further in vivo research is needed to confirm these results to advise the patients under chemotherapy to use this type of sugar.

V. FUNDING

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VI. CONFLICT OF INTEREST

All the authors declare that they have no conflict of interest.

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