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## A high glucose concentration is well tolerated by colorectal adenocarcinoma and melanoma cells but toxic to normal human gingival fibroblast: Results of an *in vitro* investigation

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### Abstract

**Background:** Glucose is associated with weight gain, which increases the risk of cancer. There is insufficient information on the effects of high glucose concentrations on cell lines. This study evaluated the viability patterns of human cancer and normal cell lines treated with glucose. **Methods:** Human gingival fibroblast (hGF), colorectal adenocarcinoma (HT29), and skin malignant melanoma (A375) cell lines were cultured and treated with additional glucose in three respective concentrations: 1 mg/ml, 5 mg/ml, and 10 mg/ml. Then, cell viability was measured using an MTT(3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide)-assay. The data were analyzed using the Statistical Package for the Social Sciences software. **Results:** The hGF cells' percentage pattern of viability showed a rapid decline of nearly 95% on the third day of treatment. Both HT29 and A375 were able to survive in the high glucose condition when the cell viability percentage was above 100% on Day 5. The data exhibited significance; the *p*-value was less than 0.001. **Conclusion:** The high glucose concentration can be toxic to hGF. In addition, HT29 and A375 might be adaptive to the hyperglycaemic condition.

*Keywords:* *in vitro* technique, fibroblasts, adenocarcinoma, malignant melanoma, glucose, cancer

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### Introduction

Physiologically, DNA quality is checked and verified by a complex set of rules and restrictions during cell division.<sup>1</sup> The control mechanism in the body can malfunction, forming cancer cells.<sup>2</sup> Cancer constitutes a large number of diseases that are characterized by abnormal cell development, which divide uncontrollably and can infiltrate and destroy normal body tissues.<sup>3</sup> Cancer pathophysiology is very complex; however, a common feature of normal and cancer cells is that both consume sugar as one of their energy sources.<sup>2</sup>

Sugar is an essential source of energy for the body's cellular activities. In association with other factors, such as a sedentary lifestyle, an uncontrolled sugary diet could be one reason behind body mass index increases.<sup>4</sup> Excessive sugar intake does not benefit the body since it may be converted into fat, causing overweight and obesity. Fat is very important since it helps maintain the production of hormones and growth factors in the body.<sup>5</sup> Nevertheless, overproduction of these hormones and growth factors will enhance cancer cell growth. Only one study has reported that high-level growth

factors can increase the risk of several cancers, such as colorectal, prostate, and breast.<sup>6</sup>

To date, no conclusive evidence has linked sugar or any monosaccharides directly to cancer; nonetheless, excessive sugar consumption may alter the body's physiology, indirectly increasing the likelihood of cancer. A variety of sweeteners or simple sugars is commercially available on the market. Excessive sugar consumption has long been associated with diabetes. However, there is a paucity of information on whether or not glucose can cause cancer, hindering scientists from identifying the best management or treatment of cancer. To seek treatment for cancer, it is crucial to have information on the behavior and pattern of cancer cell growth. This study investigated the viability pattern of human cancer and normal cell lines treated with glucose at three different concentrations which are 1 mg/mL, 5 mg/mL, and 10 mg/mL.

### Methods

**Selection and preparation of cancer and normal cell lines.** Three types of cell lines were used in this study:

human colorectal adenocarcinoma (HT-29, ATCC HTB-38, USA), human malignant melanoma (A375, ATCC CRL-1619, USA), and human gingival fibroblast (HGF-1, ATCC CRL-2014, USA).

**Selection of monosaccharide.** The monosaccharide used in this study was glucose, procured from a supplier in powder form.

**Optimization of concentration and treatment periods.**

The optimization of concentration and treatment periods of this *in vitro* stability study followed a modified version of that of Shahbuddin (2014).<sup>7</sup> Glucose was diluted properly in three different concentrations: 1 mg/mL, 5 mg/mL, and 10 mg/mL. The glucose was dissolved completely in the Dulbecco's Modified Eagle Medium (DMEM) to remove the possibility of all physical effects on the cell lines. This study's DMEM control contained only 10% of fetal bovine serum (FBS) and 1% of an antibiotic solution. In this study, four different incubation periods (1, 3, 5, and 10 days) were applied as treatment parameters of the effects of glucose on the tested cell lines' viability. The incubation time involved a process of doubling time, which ensured that the data showed the cell lines' constant growth.

**Cell culture technique.** The cells (HT-29, A375, and HGF-1) were thawed and revived in a 25-ml culture flask containing basal medium and incubated overnight at an optimum temperature of 37 °C, with 5% of CO<sub>2</sub> and 90% humidity. The basal medium used in this study was DMEM, to which were added 10% of FBS and 1% of antibiotic. The media were changed after three days of incubation to ensure that the nutrients were sufficient for cell growth and development. The cell proliferation activity was observed daily until it reached approximately 80% confluence, at which time the cells were dissociated from the media before cell seeding.

**Viability test by the MTT assay.** The MTT assay is a colorimetric technique used to assess the cells' metabolic activity. Prior to the cell viability test, a hemocytometer was used to count the cells following previous study.<sup>6</sup> Using DMEM, approximately 1,000 cells (HT-29, A375, and HGF-1) were aliquoted into different 12-well plates containing 10% of FBS and 1% of antibiotic and glucose. The 12-well plates of glucose were incubated at the four distinct intervals of 1, 3, 5, and 10 days.

The treated cell lines in the well plates were washed with phosphate buffer saline. Then, 20 µL of MTT solution, with a concentration of 0.5 mg/mL, was added to each well and incubated for at least two hours. The MTT solution was removed but then received 200 µL of dimethyl sulfoxide (DMSO) to solubilize the formazan crystals. A spectrophotometer was used to measure the

absorbance under a wavelength of 450 nm. All cell viability processes were treated as light-sensitive and wrapped in aluminum foil.

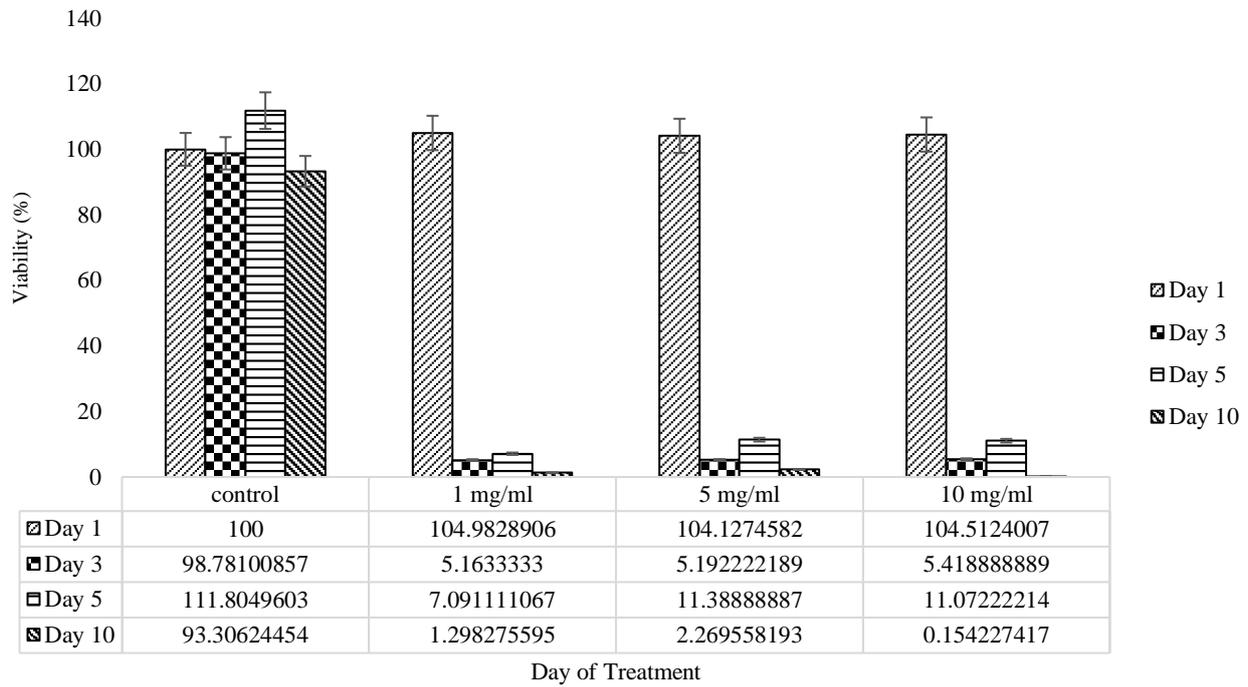
**Data analysis.** The data were analyzed using the Statistical Package for the Social Sciences software. All treated groups were compared with the control group. The values were expressed as a standard error of the mean ± standard deviation. Significant differences between the treated groups and the control were determined using one-way ANOVA. A *p*-value ≤ 0.05 was considered significant.

## Results

The cell viability percentage of the normal human gingival fibroblast (hGF) showed a rapid decline on Day 3, after treatment with glucose, whereby nearly 95% of the cells decreased from Day 1. On Day 5, the normal hGF's cell viability showed a slight increase of around 2%. Finally, the cells continued to decline until Day 10. In contrast, the control group showed a consistent pattern from Day 1 to Day 3. The control group also showed an increase above 100% on Day 5 and a decline on Day 10 (Figure 1). The value of hGF treated with glucose was less than 0.001, which is considered significant (Table 1).

The cell viability percentage of the skin malignant melanoma (A375) showed the potential to adapt when treated with higher glucose. Overall, the pattern of all treated and untreated groups showed an increase from Day 1 until Day 5 and started to decline on Day 10, except for the group treated with a concentration of 10 mg/ml. The cell viability percentage of the group treated with 10 mg/ml of glucose decreased slightly on the third day; however, it increased on Day 5 before declining on Day 10. Among the treatment groups, the highest A375 cells viability percentage (above 100%) was on Day 5 of incubation (Figure 2). The value of A375 when treated with higher glucose was less than 0.001, which is considered significant (Table 1).

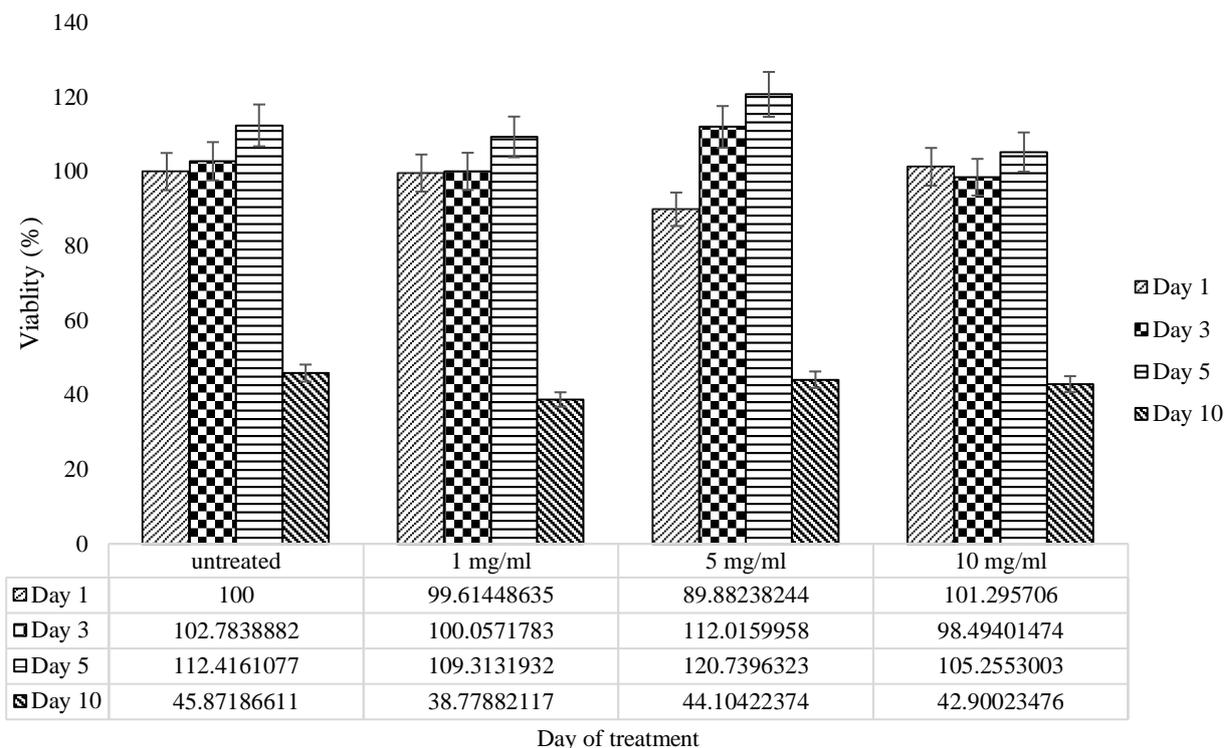
The pattern of the human colorectal adenocarcinoma (HT29) cell viability, which was treated with glucose, increased from Day 1 until Day 5, with a percentage of above 100%. On Day 10, the HT29 cells rapidly fell to 38.8%, 44.1%, and 43.9%, according to the different concentrations of 1 mg/mL, 5 mg/mL, and 10 mg/mL, respectively. In contrast, the HT29 cells in the untreated group consistently increased until Day 5 (144.5%) and leveled off on Day 10. Among the treatment groups, the highest cell viability percentage of HT29 occurred with 5 mg/mL of glucose concentration (Figure 3). The value of HT29, when treated with higher glucose, was less than 0.001, which is considered significant (Table 1).



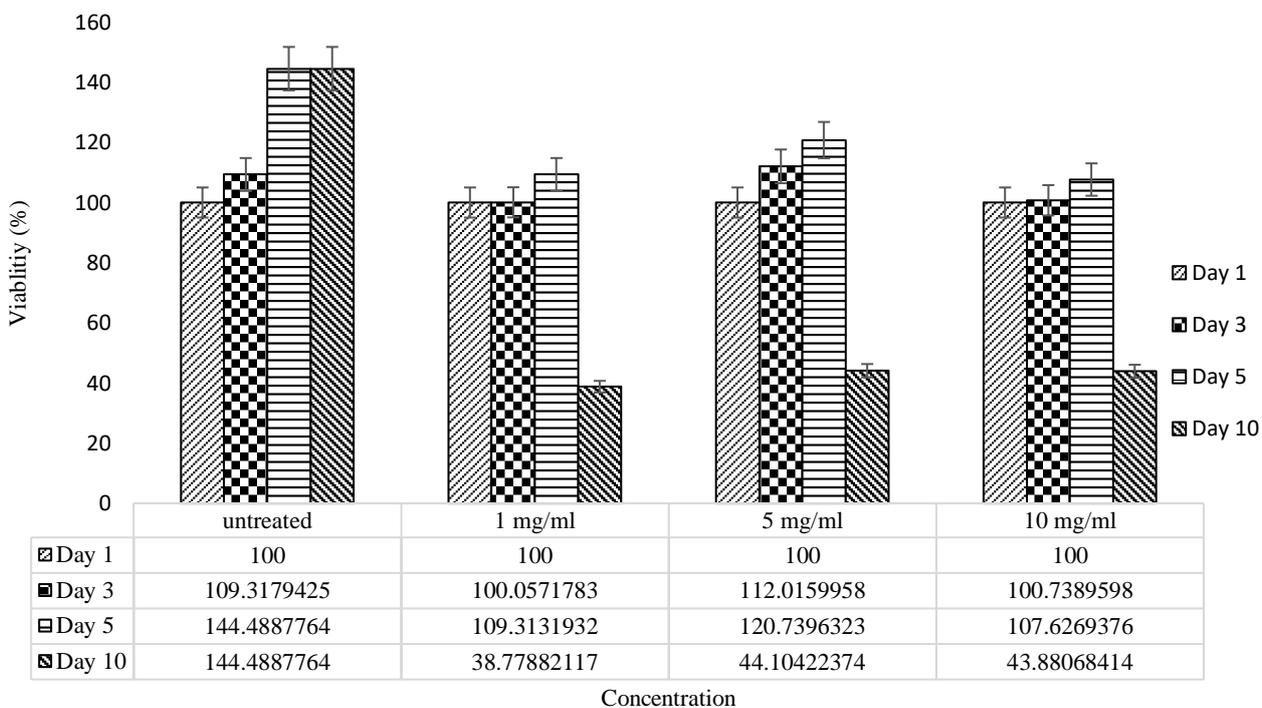
**Figure 1.** The percentage of HGF cells when treated with glucose of 1 mg/mL, 5 mg/mL, and 10 mg/mL for three intervals on Day 1, Day 3, Day 5, and Day 10

**Table 1.** One-way ANOVA table of HGF-Glucose, A375-Glucose and HT29-Glucose

	Sum of Squares	Df	Mean Square	F	p
<b>HGF-Glucose</b>					
Between groups	0.062	15	0.004	3.490	< 0.001
Within groups	0.152	128	0.001		
Total	0.214	143			
<b>A375-Glucose</b>					
Between groups	26.244	15	1.750	27.695	< 0.001
Within groups	46.748	740	.063		
Total	72.991	755			
<b>HT29-Glucose</b>					
Between groups	681621.025	15	45441.402	41.323	<0.001
Within groups	734581.303	668	1099.673		
Total	1416202.328	683			



**Figure 2.** The percentage of A375 cells when treated with glucose of 1 mg/mL, 5 mg/mL, and 10 mg/mL for three intervals on Day 1, Day 3, Day 5, and Day 10



**Figure 3.** The percentage of HT29 cells when treated with glucose of 1 mg/mL, 5 mg/mL, and 10 mg/mL for three intervals on Day 1, Day 3, Day 5, and Day 10

## Discussion

Glucose is an essential source of energy for both normal and cancer cells. It is very difficult to blame glucose as a direct cause of cancer. However, glucose may be associated with other factors, such as body weight increase and physical inactivity. For instance, a study reported that higher intake of sugar and sugar-sweetened beverages may increase the risk of type I endometrial cancers.<sup>9</sup> In this study, the patterns of both normal and cancer cells were investigated to detect any possible differences between them.

The cell viability results showed that glucose overconsumption can be toxic to the normal hGF cells. In addition, the HT29 and A375 cells could survive when treated with high glucose. The results also indicated that the normal hGF cells experienced a rapid decline after Day 1, while cancer cells (A375 and HT29) could survive with a high glucose concentration.

The mitochondria was where the glucose was processed to provide ATP, a form of energy that is important for cellular activity.<sup>10</sup> During ATP production, the free radicals, alongside their by-products, were produced.<sup>11</sup> The free radicals could induce oxidative stress, a condition that causes an imbalance between the production of free radicals and reactive oxygen species (ROS) in the body.<sup>12</sup> The effects of oxidative stress vary; for example, mild oxidative stress is temporary and tolerable, whereas chronic oxidative stress may cause critical conditions, such as cancer and diabetes, by severely damaging the structures and functions of cells.<sup>13</sup> In addition, the ROS imbalance could cause normal cells to die by either necrosis or apoptosis.<sup>14-15</sup>

In this study, the normal hGF cells were treated with higher glucose. The treated groups' pattern showed that the percentage of the hGF cell viability declined sharply from Day 3 onward. This trend could imply that the normal hGF cells do not favor a high glucose concentration, which causes cell death. Glucose metabolism in mitochondria could synthesize the protein whose function is to execute apoptosis. For example, cytochrome c is involved in glucose metabolism via the electron transport chain, which is vital to the formation of apoptosome and the progression of apoptosis.<sup>16</sup> Elsewhere, it has also been reported that high glucose-induced oxidative stress could impair hGFs' proliferation and migration.<sup>17</sup> Therefore, oxidative stress might be the factor that caused the hGF cell lines to suffer a rapid decline in incubation after Day 1.

There is a fundamental difference between normal and cancer cells. As noted earlier, the latter can grow uncontrollably and become very invasive.<sup>2</sup> The A375 and HT29 cells, which had been treated with additional

glucose, showed a similar pattern to that of the untreated group. The growth pattern of all groups of cells was, according to the four phases of lag, exponential, stationary, and death. However, the group that was treated with 10 mg/mL of glucose showed a slight decrease on Day 3. Cancer cells can be very adaptive to the high glucose-induced oxidative stress condition. A study has reported that, under the oxidative stress condition, the prostate-associated gene 4 (PAGE4) expression also increases, which subsequently avoids cancer cell apoptosis.<sup>18</sup> PAGE4 is a highly intrinsically disordered protein, whereas protein overexpression could protect the cells from stress-induced death.<sup>19</sup> Another study has reported that cancer cells can ignore the signals that are important for preventing cell division or the beginning of a process known as "programmed cell death," whereby the body tends to dispose of its abnormal cells by increasing CD47 production.<sup>2</sup> The CD47 protein is also known as integrin-associated protein, which is encoded by the CD47 gene.<sup>20</sup> This CD47 protein will avoid normal phagocytosis by binding to the receptor signal-regulatory protein- $\alpha$  (SIRP- $\alpha$ ) in macrophages.<sup>21</sup> Hence, this development could explain why the cancer cell lines managed to survive in the higher glucose condition.

## Conclusion

In conclusion, high glucose concentration can be toxic to hGF cells. Also, the HT29 and A375 cells have the potential to adapt to the hyperglycaemic condition.

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## Conflict of Interest Statement

The authors declare no conflict of interest.

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