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Assessing The Efficacy of Dextran And Dextransucrase In Modulating MCF-7 Breast Cancer Cell Activity

[1]Yasameen Hasan Ali
[2]Sahira Nsayef Muslim
[3]Israa Hussain Hamzah

[1,2,3]Department of Biology, College of Science, Mustansiriyah University, Baghdad/ Iraq

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**Corresponding Author:*

Emails:
yasameenhasan75@gmail.com

Abstract: *Tumor development is a complex process involving abnormal cell proliferation within an organism. This study aims to understand tumors, their categorization, formation methods, and effects on human health. Tumor growth is influenced by genetic, environmental, and lifestyle factors. Dextran, a glucose-based polysaccharide, has been used in medical applications for blood plasma substitutes and antithrombotic agents. Researchers are interested in its potential therapeutic applications due to its biocompatibility and unique properties. Dextransucrase, a glucosyltransferase, plays a vital role in synthesizing glucan polymers. Advancements in cancer research reveal the nuanced roles of biopolymers like dextran in tumor biology, drug delivery, and cancer therapy. In this study MCF-7 cell line maintained in MEM, reseeded twice a week, incubated at 37°C. The MTT cell viability assay was conducted on 96-well plates to assess cytotoxic effects. Cell lines were seeded, treated with the tested compound, and measured after 72 hours. After solubilization, absorbency was determined using a microplate reader at 492 nm. The results showed A decrease in cell viability was observed with decreasing Dextran concentration, with a negative correlation between concentration and cell viability. A P-value of 0.001 indicates statistically significant effects on MCF-7 cell cytotoxicity, rejecting the null hypothesis that Dextran has no effect. Dextransucrase exhibited a dose-dependent cytotoxicity effect on MCF-7 cells, with a significant effect at the highest concentration (1000) and a negative correlation at the lowest concentration (31.25). The P-value of 0.001 was below the 0.05 threshold, rejecting the null hypothesis. According to the results of cell viability, the highest concentration (1000) had the lowest viability, while the lowest concentration (31.25) had the highest. The data supports the cytotoxic potential of Dextransucrase in a dose-dependent manner.*

Keywords: angiogenesis, cytotoxic, IC50, Neoplasms and viability,MEM.MCF-7

1.Introduction

The process of developing a tumor is intricate and multidimensional, and it includes the abnormal proliferation of cells inside an organism. An overview of tumors, their categorization, methods of formation, and effects on human health are the goals of this scholarly study [1]**.** Neoplasms, often known as tumors, are abnormal cell growths that develop as a result of aberrant cell differentiation and division [2]. They may develop in different human tissues and organs, which can result in a broad variety of illnesses, including cancer. A complicated field of research, tumor growth is impacted by genetic, environmental, and lifestyle variables [3]**.**

Numerous genetic and epigenetic changes that interfere with the normal control of cell growth and division result in the formation of tumors. These changes may be inherited or acquired during the course of a person's lifetime. Specific gene mutations, such as those in oncogenes and tumor suppressor genes, are crucial in triggering and fostering the development of tumors. The deregulation of gene expression in tumors may also be attributed to epigenetic alterations, such as DNA methylation and histone modifications [4]**.** Different cellular and non-cellular elements that interact with tumor cells make up the tumor microenvironment. Tumor growth and development are facilitated by extracellular matrix elements, blood arteries, fibroblasts, and immune cells. The tumor microenvironment may encourage angiogenesis, dampen immunological responses, and provide a favorable setting for the growth of tumor cells [5]**.**

Dextran, a complex polysaccharide primarily composed of glucose molecules, has historically been utilized in various medical applications ranging from blood plasma substitutes to antithrombotic agents due to its biocompatibility and unique physicochemical properties [6]. Produced by bacteria via the enzymatic action of dextransucrase on sucrose, dextran has piqued the interest of researchers for its potential therapeutic applications. Dextransucrase, in its right, belongs to a family of glucosyltransferases that play a vital role in synthesizing these glucan polymers [7]. Over the years, advancements in cancer research have unveiled the nuanced roles of various biopolymers, including dextran, in tumor biology, drug delivery, and cancer therapy. The coupling of these insights with the modifiable nature of dextran has opened up avenues for its potential application in targeted drug delivery, immunomodulation, and even direct therapeutic roles in cancer management [8].

MCF-7 was established in 1973 at the Michigan Cancer Foundation (from which it gets its name) by Herbert Soule and colleagues. It was derived from the metastatic pleural effusion of a 69-year-old Caucasian woman with invasive ductal carcinoma [9]. MCF-7 is hormone-responsive, expressing both estrogen and progesterone receptors (ER and PR), which is reflective of a significant subset of human breast cancers. It also exhibits epithelial morphology and forms well-differentiated adenocarcinomas when implanted in immune-deficient mice (Levenson and Jordan, 1997). However, unlike many breast tumors, MCF-7 cells do not overexpress HER2, an oncogenic receptor tyrosine kinase [10]. The MCF-7 cell line has been instrumental in understanding the biology of breast cancer. Its hormone receptor-positive status has made it a model of choice for studying the mechanisms of hormone action and resistance in breast cancer. It has significantly contributed to the development of hormonal therapies, like tamoxifen and aromatase inhibitors [11]. MCF-7 cells are also widely used in drug screening and cytotoxicity assays. They have been employed to assess the efficacy and toxicity of novel anti-cancer drugs and to study drug resistance mechanisms [12]. Furthermore, MCF-7 has been used in

metastasis studies, despite its relatively low metastatic potential. Transfection with appropriate oncogenes or exposure to a hypoxic environment can enhance its invasiveness, enabling studies of metastatic processes [13].

In this research, delves into the therapeutic potential of dextran and dextransucrase, specifically assessing their impact on the MCF-7 breast cancer cell line, thereby providing insights into their possible roles in breast cancer management.

2.Methodology

Maintenance of cell cultures

MCF-7 cell line, was maintained in MEM supplemented with 10% Fetal bovine, 100 units/mL penicillin, and 100 µg/mL streptomycin. Cells were passaged using Trypsin-EDTA reseeded at 50% confluence twice a week, and incubated at 37 °C.

Cytotoxicity Assays

To determine the cytotoxic effect, the MTT cell viability assay was conducted on 96-well plates. Cell lines were seeded at 1 104 cells/well. After 24 hrs. or a confluent monolayer was achieved, cells were treated with the tested compound. Cell viability was measured after 72 h of treatment by removing the medium, adding 28 L of a 2 mg/mL solution of MTT, and incubating the cells for 1.5 h at 37 °C. After removing the MTT solution, the crystals remaining in the wells were solubilized by the addition of 130 L of DMSO (dimethyl sulfoxide), followed by 37 °C incubation for 15 min with shaking. The absorbency was determined on a microplate reader at 492 nm (the test wavelength); the assay was performed in triplicate.

The inhibition rate of cell growth (the percentage of cytotoxicity) was calculated as the following equation: -

% Cell viability = (Absorbance of treated cell / Absorbance of non-treated cell) x 100

% Cytotoxicity = 100 – cell viability

Statistical analysis:

The obtained data were statically analyzed using an unpaired t-test with SPSS. The values were presented as the mean ± SD of triplicate measurements.

Results and Discussion

A dose-dependent decrease in cell viability was observed as the concentration of Dextran decreased, as shown in figure (1). At the highest Dextran concentration (1000), cytotoxicity was recorded at 44.61, while the lowest concentration (31.25) yielded a cytotoxicity level of 1.49. A consistent decrease in cytotoxicity with a reduction in Dextran concentration was recorded, indicating a negative correlation between Dextran concentration and cell viability. A P-value of 0.001 was obtained from the analysis, significantly below the standard threshold of 0.05. This suggests that the observed effects of Dextran on MCF-7 cell cytotoxicity are statistically significant, warranting rejection of the null hypothesis (i.e., Dextran has no effect on the cytotoxicity of MCF-7 cells).

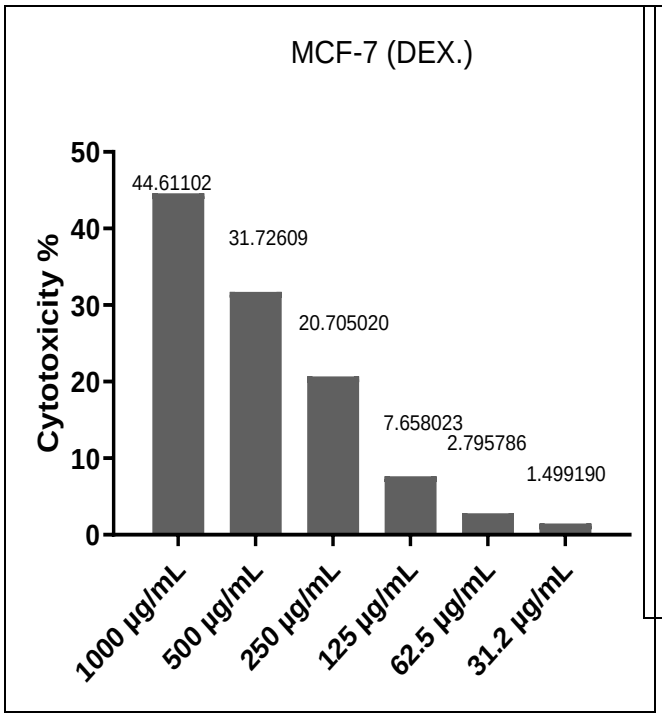


Figure (1); percentage of cytotoxicity corresponding to the concentration of Dextran.

Similar to Dextran, a dose-dependent cytotoxicity effect was observed with Dextransucrase as well, as shown in figure (2). At the highest concentration (1000), cytotoxicity was recorded at 37.51, and at the lowest concentration (31.25), cytotoxicity was at 4.95. A decrease in cytotoxicity was observed with a reduction in the Dextransucrase concentration, indicative of a negative correlation.

The P-value of 0.001 obtained from the Dextransucrase experiments also falls well below the conventional threshold of 0.05. This suggests a statistically significant cytotoxic effect of the Dextransucrase on MCF-7 cells, thus rejecting the null hypothesis that the Dextransucrase does not affect MCF-7 cytotoxicity.

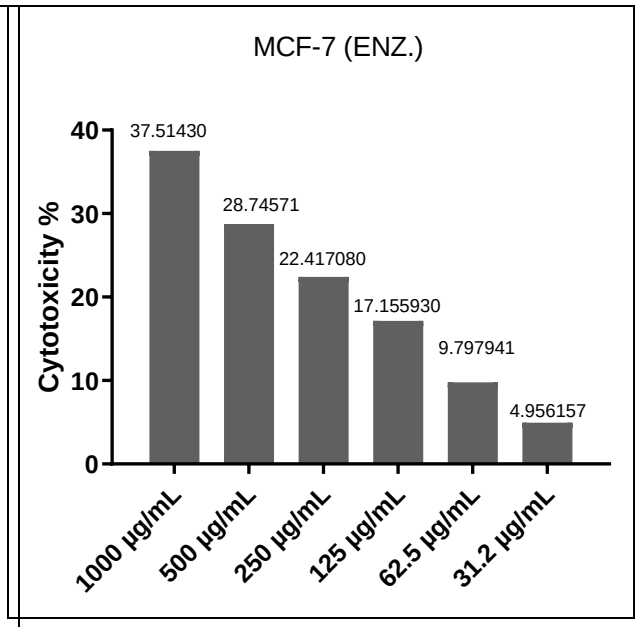


Figure (2); percentage of cytotoxicity corresponding to the concentration of Dextransucrase.

The IC50 value for Dextran in the MCF-7 cells was found to be 595.5, indicating that this is the concentration at which Dextran reduced cell viability by 50%, as shown in figure (3). Conversely, the IC50 value which are shown in figure (4) for the Dextransucrase was determined to be 258.3, representing a lower concentration needed to achieve a similar reduction in cell viability.

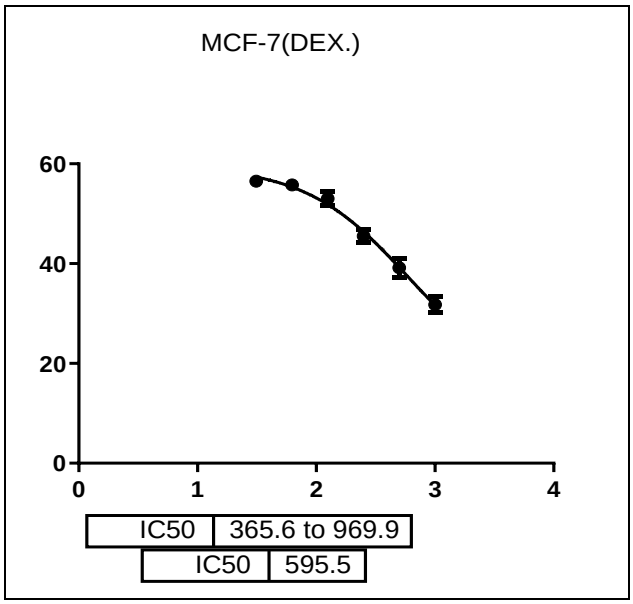


Figure (3); IC50 of dextran compound on Michigan Cancer Foundation-7

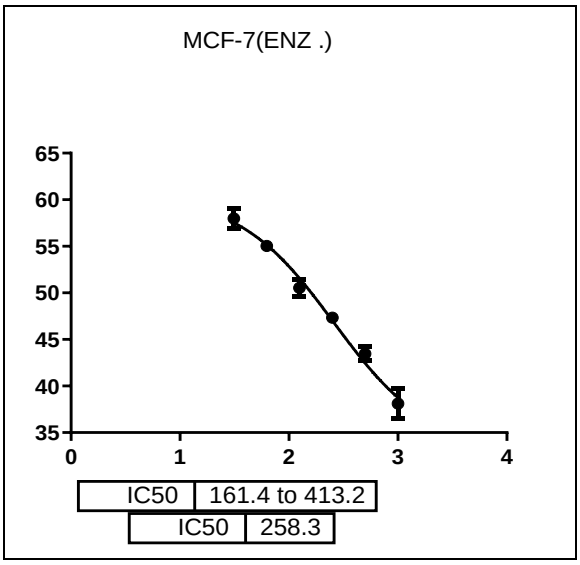


Figure (4); IC50 of dextransucrase compound on Michigan Cancer Foundation-7

The results reflected a decrease in cell viability correlating with an increase in Dextran concentration, signifying Dextran's cytotoxic impact on MCF-7 cells, as shown in figure (5). At the highest concentration of Dextran (1000), cell viability was significantly compromised, presenting the lowest value at 4.2. Conversely, the cell viability was closest to the untreated control (8.1) at the lowest Dextran concentration (31.25), which showed a cell viability of 8. These findings were statistically significant, as indicated by a P-value of 0.001, considerably below the conventional threshold of 0.05. This result supports the hypothesis that Dextran concentration negatively impacts the viability of MCF-7 cells.

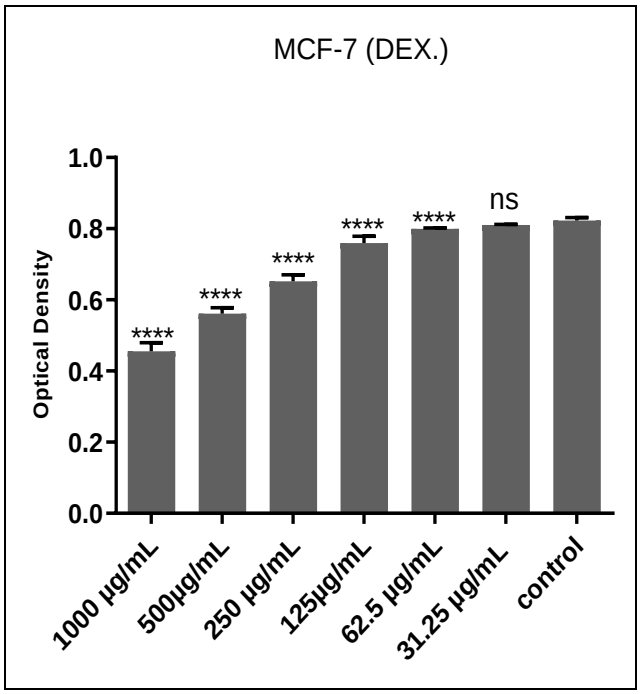


Figure (5); cell viability represented on the Y-axis as optical density corresponding to the concentration of Dextran.

The investigation of the Dextransucrase's impact on the viability of MCF-7 cells was also performed. The results again demonstrated a relationship between the Dextransucrase concentration and cell viability shown in figure (6), reinforcing the enzyme's cytotoxic potential. At the highest concentration of the Dextransucrase (1000), cell viability was observed to be at its lowest value of 5.3. At the lowest concentration of the Dextransucrase (31.25), cell viability was observed to be 8, which is relatively close to the cell viability observed in the untreated control sample (8.3). The data yielded a P-value of 0.001,

significantly below the conventional threshold for statistical significance (0.05). This strongly supports the hypothesis that the Dextransucrase negatively impacts MCF-7 cell viability in a dose-dependent manner.

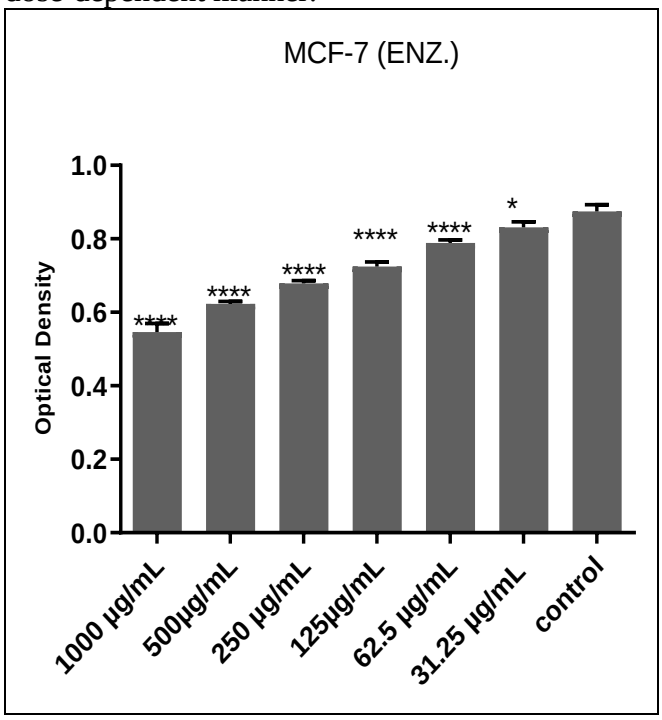
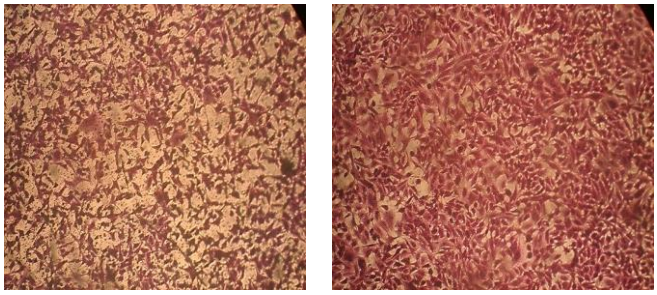
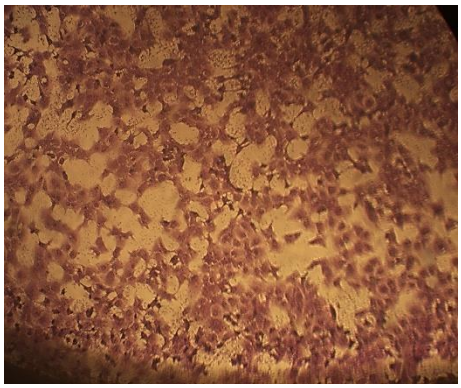


Figure (6); cell viability represented on the Y-axis as optical density corresponding to the concentration of Dextransucrase.



MCF-7 (Dextran treated) (Control)



MCF-7 (Enzyme treated)

Figure (7); Effect of dextran and dextransucrase on MCF-7 cells.

The results showed a clear dose-dependent cytotoxic effect for both dextran and the dextransucrase, whereby a decrease in the concentrations of these compounds resulted in reduced cell toxicity. Specifically, at the highest dextran concentration of 1000, cytotoxicity was recorded at 44.61, and at the lowest concentration of 31.25, cytotoxicity was significantly reduced to 1.49. Similarly, the dextransucrase also showed a dose-dependent cytotoxic effect, with cytotoxicity at the highest concentration of 1000 recorded at 37.51 and at the lowest concentration of 31.25 recorded at 4.95. These findings suggest that dextran and the dextransucrase have a cytotoxic effect on MCF-7 cells, with this cytotoxicity decreasing as the concentration of these compounds decreases. The higher cytotoxicity of dextran at comparable concentrations implies it may be more potent in its cytotoxic effects compared to the dextransucrase. This further strengthens the potential therapeutic utility of dextran, which might function not only by inhibiting angiogenesis, as previously discussed, but also by directly inducing cell death in cancer cells. These results disagreed with a previous study that showed that blank dextran polymeric micelles had no significant effect on MCF-7 cell proliferation and its safety range was determined 0.00001- 100 µM [14]. And also disagreed with another previous study, that

showed Dex-SA carrier has no significant effect been observed in MCF-7 cell lines [15].

The IC50 values, or the half maximal inhibitory concentration. In this context, the IC50 values were used to compare the cytotoxic potency of dextran and the dextransucrase on MCF-7 cells. The IC50 value for dextran was determined to be 595.5. This suggests that a higher concentration of dextran is required to achieve a 50% reduction in cell viability, indicating its cytotoxic effects on MCF-7 cells. Conversely, the IC50 value for the dextransucrase was found to be lower, at 258.3. This lower IC50 value implies a higher cytotoxic potency compared to dextran, as less dextransucrase is needed to achieve the same reduction in cell viability.

Both dextran and dextransucrase may exert their cytotoxic effects by directly interacting with the cell membrane. The polysaccharide nature of dextran allows it to interact with membrane proteins or lipids, disrupting the membrane integrity and leading to cell death [16]. Dextran's greater cytotoxic effect observed in this study might be attributed to its stronger or more specific interactions with the membrane components compared to the dextransucrase.

The observed cytotoxic effects might also result from the induction of apoptosis, a type of programmed cell death. Dextran and dextransucrase could potentially trigger various intracellular signaling pathways that lead to apoptosis, such as the mitochondrial pathway or the death receptor pathway. The higher cytotoxicity of dextran might indicate that it's more efficient at triggering these apoptotic pathways [17]. Another potential mechanism could involve the inhibition of critical metabolic processes within the MCF-7 cells. Dextran and dextransucrase could interfere with cellular functions such as protein synthesis, energy production, or DNA replication, ultimately leading to cell death. The higher cytotoxicity of dextran might be a result of it more effectively or broadly inhibiting these metabolic processes. Interference with DNA replication can lead to DNA damage or replication stress, triggering cellular responses that could result in cell cycle arrest or cell death [18].

Dextran and dextransucrase could also potentially exert their cytotoxic effects by modulating the immune response. They might stimulate the immune system to recognize and attack the cancer cells, resulting in cell death. The difference in cytotoxicity between dextran and dextransucrase could be related to how effectively they stimulate this immune response [19].

In this research, the cytotoxic effects of dextran and the dextransucrase were further studied by examining their impacts on cell viability in MCF-7 cells. Both dextran and the dextransucrase showed a clear concentration-dependent impact on cell viability, echoing their dose-dependent cytotoxic effects observed earlier.

Some studies have shown that dextran and its derivatives can have anti-angiogenic and anticancer properties. For example, research has demonstrated that certain types of dextran sulfate can inhibit angiogenesis and exhibit cytotoxic effects against various cancer cells, including breast cancer cells like MCF-7 [20]. This is in line with your study's findings, which demonstrated that dextran could inhibit angiogenesis and reduce cell viability in MCF-7 cells.

Conversely, other studies might have reported that dextran and dextransucrase don't exhibit significant cytotoxic effects on cancer cells. For instance, some researchers have suggested that while dextran might exert some anti-angiogenic effects, it does not directly cause cancer cell death [21]. Others have suggested that the effects of dextran and dextransucrase might be highly dependent on their specific molecular weight or structure [22], meaning that not all forms of these compounds would necessarily have the same effects on angiogenesis or cell viability.

4.Conclussion

The results showed A decrease in cell viability was observed with decreasing Dextran concentration, with a negative correlation between concentration and cell viability. A P-value of 0.001 indicates statistically significant effects on MCF-7 cell cytotoxicity, rejecting the null hypothesis that Dextran has no effect. Dextransucrase exhibited a dose-dependent cytotoxicity effect on MCF-7 .The P-value of 0.001 was below the 0.05 threshold, rejecting the null hypothesis. According to the results of cell viability, the highest concentration (1000) had the lowest viability, while the lowest concentration (31.25) had the highest. The data supports the cytotoxic potential of Dextransucrase in a dose-dependent manner.

Ethical Approval

The specimens of this study took approval the law and directives of the human rights organizations with adequate information in an ethical manner

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