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ANTITUMOR ACTIVITY OF DEXTRAN PRODUCED FROM LOCALIZED *LEUCONOSTOC MESENTEROIDES* ISOLATES

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ABSTRACT : In past decade, breast cancer prevention research has made notable steps forward and many evidences suggest the potential effects of natural compound in decreasing the risk of various cancers. So, in this study dextran consider as one of the medically important natural compound isolated from *Leuconostoc mesenteroides* isolate. Now today, Dextran used alone or in combination with other compound or synthesized as nanoparticle to be used as antitumor agent. In this study, the aptitude activity of dextran against MCF-7 cell lines was assessed using MTT assay in which the results indicated a crucial role of dextran in breast cancer withen IC50 value of 29.04 and all the results obtained then by using High Content Screening (HCS) explained a promising cancer treatment by dextran by decreasing cell viability and increase nuclear intensity and increase cytochrome C releasing in comparison with un treated cells in a dose dependant pattern.

Key words : Dextran, MTT assay, MCF-7, cytochrome C.

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INTRODUCTION

Dextran is a homopolysaccharide, as it consists of several glucose units that are linked together by α1,6 glycosidic bonds and constitute more than 50% of the total links with branched links with α1,3 and α1,4 or α1 bonds (Morris and Harding, 2009).

The exact chemical composition of dextran depends on the type of microbial strain that produces it and the type of the enzyme Dextransucrase that contributes to its synthesis. Among the most important producing bacterial genera are: Leuconostoc, Lactobacillus Streptococcus, Saccharomyces and the enzyme varies according to the type of the microorganism producing it, as it is of the continuous type in the strains Streptococcus bacteria, while in Leuconostoc bacteria strains of induced enzymes (Caggianiello *et al*, 2016).

Dextran is involved in a number of food industries as well as pharmaceutical industries and a number of substances of commercial importance and from the microorganisms known to produce dextran are *Leuoconostoc mesenteroides* (Lm), whose cells are spherical or lenticular in the form of short chains or pairs of cells, positive for a cram dye, the optimum growth temperature is 25-30°C of the type of differential fermentation (Juvonen *et al*, 2015; Kareem and Salman, 2019).

MATERIALS AND METHODS

Extraction of dextran from *Leuoconostoc mesenteroides*

An *Leuoconostoc mesenteroides* (Lm) bacterial isolate obtained in a previous study so it was used as ready to use compound (Ahmaed, 2014) was used as an efficient isolate in the production of dextran, and the optimum conditions for production were studied in a previous study also. Thermal yeast, 24-hour fermentation time, initial pH of medium 6.0, primary inoculum volume 1×10^6 cells/ml and temperature of 25°C in a static fermentation system achieved a dextran production of

approximately 7.7 g dry weight.

Assessment of cytotoxic activity of dextran

In order to evaluate the cytotoxicity of dextran, MTT colorimetric was assumed to determine the cytotoxic effect of dextran on breast cancer cell lines MCF-7 (Freshney, 2010). The MCF-7 cells (1×10^4–1×10^6 cells mL^{-1}) were seeded on 96-well micro-titer plates to reached final volume of 200 µL and then cultured at 37°C for 24 h. Following incubation for 24 h, serial dilutions of dextran ranged from 6.25 to 400 µg/ml were added and triplicates were used for each concentration as well as the controls (cells treated with serum free medium). 10 mL of MTT solution was added, followed by incubation at 37°C, 5% -CO_2 for 4 h. After carefully removing media, 100 mL of solubilisation solution was added to each well and incubated for 5 min. Formation of formazan was determined by measuring the absorbance at 570 nm using an ELISA microplate reader (Bio-Rad, USA).

High-content screening assay

Procedure of Palaniswamy *et al* (2012) was followed in which a kit from Thermo Scientific Cellomics multi-parameter cytotoxicity 3 kit (Thermo Scientific, Japan) was used in this study (Cytochrome c used as a primary antibody in addition to other components including DyLight™ 649 conjugated goat anti-mouse IgG, Hoechst dyes, wash buffer (10× Dulbecco's phosphate buffered saline [PBS]), permeabilization buffer (10× Dulbecco's PBS with 1% Triton® X-100) and a blocking buffer (10×)) was used for the immediate recognition of cell viability, nuclear intensity and cytochrome C level in the MCF7 breast cancer cell line. The distribution and intensity of fluorescence within cells were imaged ($n = 5$) (Thermo Scientific, Japan) by using an HCS system. Theses system was attached to a computerized imaging microscope (with a Zeiss 40× (0.75 NA)). The mitochondrial membrane potential (MMP) and the cell permeability dyes were added to cells treated with tamoxifen for 24 h and incubated for 30 min at 37°C. Finally, Hoechst 33342 staining assay in which Cell nuclear morphology changes of MCF7 cells that cultured in 6-well plates for 24 h detected using a Zeiss Axio Observer microscope (Thermo Scientific, Japan).

Statically analysis

The values of the investigated parameters were given in terms of mean ± standard deviation (SD) and differences between means were assessed by analysis of variance (ANOVA) followed by least significant difference (LSD) or Duncan test, using the computer GraphPad Prism version 6 (Graph Pad Software Inc.).

RESULTS AND DISCUSSION

A. The test of 3-(dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) was accomplished to conclude the cytotoxic effect of *dextran extract* on breast cancer cell line (MCF-7). MTT Assay was made to calculate the cell viability and inhibition rate on the tumor cell line by using different concentration of dextran. The cytotoxic effect evaluated at concentrations ranged from 6.2-400 ìg/ml as shown in Table 1. The results represented a decrease in cell viability in a dose-dependent pattern. The cell viability is reduced by increasing dextran concentrations. The maximum decreasing in MCF-7 cell viability (%) was noted by 400µg/ml (44.45±1.20) while the highest MCF-7 cell viability at 6.2 µg/ml reached (94.52±0.57). The dextran exhibited significantly the most potent cytotoxic activity with IC_{50} value of 29.04 µg/ml. However, an IC_{50} of 99.39 µg/ml was obtained from the effect of dextran on WRI-68 normal cell line (Fig. 1).

B. Results of high content screening

All results indicated in Table 2 as shown below :

Cell viability : Table 2 and Fig. 2 indicated that a reduction in MCF7 cell viability increased in a dose dependant pattern by increasing the concentration of dextran. The reduction ranged from (986±0.78 to 2167±2.11) at concentrations from (400 to 6.25 µg/mL), respectively in compared to un treated cells (2780±3.01).

Nuclear intensity : The results of nuclear intensity showed that MCF7 cell nuclear intensity significantly increased to (678±17.21) after treatment with 400 µg/mL of dextran in comparison to untreated cells (435.50±34.65) and the increasing was dose dependant (Table 2, Fig. 3).

Cytochrome C : Table 2 and Fig. 4 represent that the intensity increased to (523±78.1, 488.0±75.3, 482.0±75.0, 478.0±92.3, 461.0±37.8, 461.0±31.1 and 458±29.8) for (400, 200, 100, 50, 25, 12.5 and 6.25 µg/mL) respectively in comparison to untreated cells (421.0±87.7).

Dextran known as commercially available bacterial exopolysaccharide (EPS) with possess several industrial applications in the food industry and in the biomedical industry. The importance of cell viability test that it's directly associated with the toxic effects of any compounds (Al-Jailawi *et al*, 2015). The result showed a reduction of cell viability in MCF7 cells treated with dextran could be attributed to cytostatic and/or cytocide effects by down-regulate telomerase activity (Ajabnoor *et al*, 2012). Telomerase is a diagnostic and therapeutic

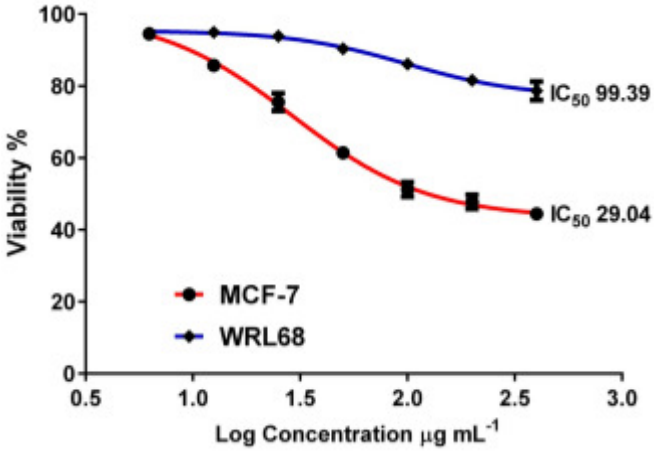

Fig. 1 : Cytotoxicity effect of dextran extract on MCF-7 and WRI-68 cells after incubation at 37ºC for 24 hours.

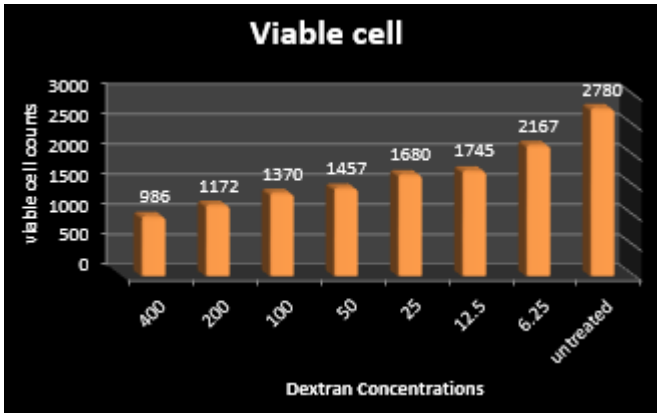

Fig. 2 : Viable cell counts after treated with different concentrations of dextran.

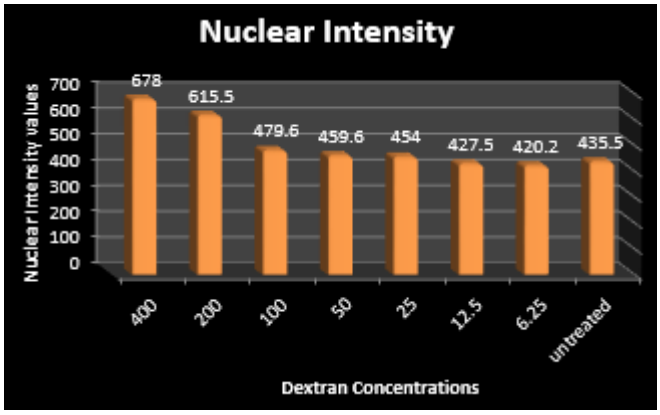

Fig. 3 : Nuclear intensity after cells treated with different concentrations of dextran.

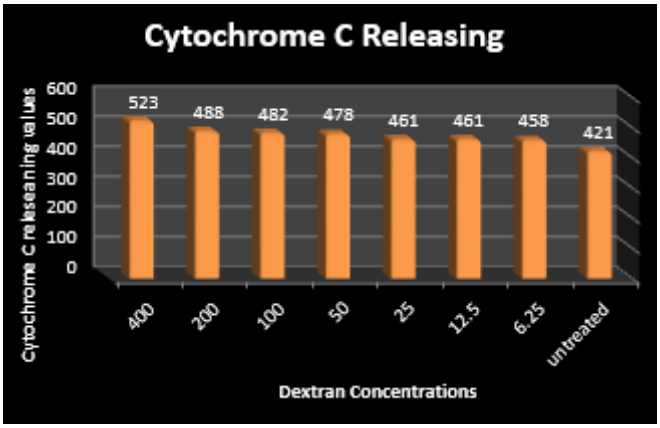

Fig. 4 : Cytochrome C releasing from cells after treated with different concentrations of dextran.

Table 1 : Cytotoxicity effect of dextran extract on MCF-7 and WRI-68 cells after 24 hours incubation at 37ºC.

Dextran concentrations (µg/mL)	Viable cell count of MCF-7 cell line Mean± S.D.	Viable cell count of WRL-68 cell line Mean± S.D.
400	44.45±1.20	78.66±2.54
200	47.80±1.94	81.64±1.43
100	51.23±2.03	86.15±1.27
50	61.45±1.36	90.39±1.30
25	75.50±2.45	93.90±1.13
12.5	85.76±1.45	94.98±0.67
6.25	94.52±0.57	94.75±0.96

Table 2 : Cytotoxicity effect of dextran on multi cellular parameters after 24 h. of incubation at 37ºC.

High content screening parameters (mean±S.D.)			
Dextran concentrations µg\ml	**Cell viability**	**Nuclear intensity**	**Cytochrome C releasing**
400	986±0.78	678±17.21	523±78.1
200	1172±1.52	615.53±14.85	488.0±75.3
100	1370±1.78	479.61±33.23	482.0±75.0
50	1457±1.89	459.69±38.89	478.0±92.3
25	1680±2.13	454.00±50.91	461.0±37.8
12.5	1745±2.34	427.50±24.75	461.0±31.1
6.25	2167±2.11	420.22±25.21	458±29.8
Untreated cells	2780±3.01	435.50±34.65	421.0±87.7

biomarker as it is absent from most somatic cells and is present in most cancer cells. Telomerase synergistically with natural products may play a decisive role in the development of a drug for cancer therapy for the new decades (Anirudhan, 2016). Recent research clearly demonstrates that the impacts of natural compounds inhibit telomerase activity, inhibit cell proliferation, reduce hTERT mRNA and protein and subsequently promote apoptosis in various cancer cell lines. Researchers further suggest that natural products alter telomerase activity by suppression at transcriptional and post-transcriptional levels (Guo *et al*, 2006). The results showed the possible relationship between natural products and working regulation of telomerase and its various binding proteins to inhibit the telomere/telomerase complex (Yu *et al*, 2007). Different studied had shown that used as anti-apoptotic and pro-survival cellular pathways. Depending on its molecular weight to prolong the life of cells and

inhibits apoptosis by DNA fragmentation delay and decrease of annexin V-labeled cells, causes a G0/G1 cell cycle arrest, decreases p53 expression and increases the pro-survival factor Hsc70 expression (Abdalah *et al*, 2018). These findings suggest that a better understanding and manipulation of using dextran alone or after synthesizing the bio-pharmaceutical industry (Menvielle *et al*, 2013). Also, dextran promising anti-cancer activity by boost without any pre-modification due to their unique structural properties because its ability to conjugates assume different cellular and nuclear internalization pathways and could enhance DNA cleavage (Wang *et al*, 2013).

In summary, dextran possess dual function as a targeted anti-cancer drug carrier and DNA cleavage activity enhancer, which should be potentially useful in cancer therapy. Finally, dextran like other antitumer compound enter to tumer cell by endocytosis and are cleaved to free form by lysosomal enzyme (Tarvirdipour *et al*, 2016).

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