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Investigation of the effect of the probiotic extracted from Bifidobacterium bifidum on the HRT-18 cell line for colorectal cancer

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Abstract

The current study aimed to investigate the effect of the biosurfactant extracted from *Bifidobacterium bifidum* on the colorectal cancer cell line HRT-18. five different concentrations of the biosurfactant (BS) extracted from Bacteria *Bifidobacterium bifidum* were prepared: (400, 200, 100, 50, 25) µg/ml. The cytotoxic effects were assessed using the MTT assay, a test commonly used in cell culture , The activated cancer cells were exposed to the five prepared concentrations. The results showed cytotoxic effects of the (BS) on the HRT-18 cell line , as a decrease in the number of cancer cells was observed alongside an increase in the inhibition rate with higher concentrations. The highest inhibition rates were achieved at concentrations of (400,200) µg/ml The percentages were 68.4,61.3% respectively. Meanwhile the (BS) did not exhibit significant cytotoxicity on normal HdFn cells at concentrations of (400,200) µg/ml. The results showed significant differences at P≤0.0001 in IC50 when treating HRT-18 cancer cells with the biosurfactant (BS) where it was 53.4 µg/ml. For the normal HdFn cell line, the IC50 was 218.2 µg/ml. The results of the MTT assay showed inhibition when treating the colorectal cancer cell line HRT-18 and the normal cell line with the chemotherapy drug FOLFOX at different concentrations. The IC50 calculation results for the HRT-18 cancer cells were 77.1 µg/mL, and for the normal HdFn cell line, it was 174.6 µg/ml. The IC50 concentration results for treating the HRT-18 cancer cell line with the biosurfactant (BS) were lower compared to the chemotherapy drug FOLFOX, which is used as an effective treatment for colorectal cancer. The BS demonstrated lower cytotoxicity against normal HdFn cells compared to the chemotherapy drug FOLFOX. The results confirmed significant differences at P<0.0001 between (BS) and FOLFOX in the HRT-18 cancer cell line at concentrations of (400, 200, 100, 50, 25) µg/ml. Meanwhile, the results showed significant differences between (BS) and FOLFOX in the normal HdFn cell line at concentrations of (400, 200) µg/mL, but no significant differences were observed at concentrations of (100, 50, 25) µg/ml.

***Keywords*:** *Bifidobacterium bifidum , biosurfactants Probiotics , Cytotoxicity , FOLFOX , MTT Assay .*

1. INTRODUCTION

Biosurfactants are biological substances produced by various microorganisms, including *B. bifidum* which compound reduces the surface tension between liquids. They are considered antimicrobial and anti-adhesive agents [1]. *B. bifidum* are considered probiotic bacteria that demonstrate potential in producing biosurfactants. These bacteria are regarded as safe for use in the food and pharmaceutical industries [2]. Biosurfactants function with a mechanism similar to that of surfactants and play a key role in various processes such as emulsification, de-emulsification, wetting, foaming, polymerization, and phase dispersion [3]. These substances are non-toxic, biodegradable, and environmentally friendly, with selective microbial surface properties , They are characterized by long-term physicochemical stability, can be produced on a large scale, and withstand harsh environmental conditions. This makes them suitable for a wide range of applications [4].

HRT-18 cells are one of the crucial Colorectal cancer cellular lines used in medical research. These cells had been remoted from a human colon most cancers tumor and are widely used to study the characteristics of colon most cancers and broaden new treatments [5]. The cancer cell line HCT-8 is highly similar to the HRT-18 cancer cell line in terms of morphological and genetic patterns. It is suspected that the colorectal cancer cell lines HCT-8 and HRT-18 are essentially identical

[6].The HRT-18 also known as HCT-8 is a vital cell line for exploring the fundamental mechanisms and potential treatments for adenocarcinoma of the colon , Derived from human colon tissues HRT-18 holds a significant position in colorectal cancer research , Scientists utilize this cell line to gain insights into tumor biology, investigate molecular pathways , and develop new therapeutic strategies , making it a valuable tool in advancing research on adenocarcinoma and colorectal cancer as a whole [7].

2. MATERIALS AND METHODS

2.1. Maintenance and preparation of the HRT-18 cell line

To activate and prepare the cancer cell line, I relied on the method [8].The preparation of RPMI-1640 culture medium , is used for cell manner of life applications , It typically includes dissolving the RPMI-1640 powder in distilled water, adjusting the pH to the popular degree, sterilizing the medium via filtration, and supplementing it with critical components like fetal bovine serum (FBS), antibiotics, or unique growth factors primarily based mostly on the particular requirements of the cell line being cultured.

2.2. Refrigeration of cell line

The HRT-18 cell line was frozen using a cryopreservation medium consisting of serum-free medium (SFM), fetal bovine serum (FBS), and DMSO in specific proportions. Precise procedures must be followed during freezing to ensure cell quality preservation [9]. It is recommended to prepare the freezing medium freshly at the time of initiating the cryopreservation process for optimal results.

2.3. Preparation of different concentrations of (BS) and the chemotherapy FOLFOX.

Different concentrations of the biosurfactant (BS) and the chemotherapy drug (FOLFOX) were prepared. A total of 0.01 g of the dry extract for each compound was dissolved in serum-free medium (SFM), followed by serial dilution to create five half-diluted concentrations, down to the lowest concentration: 400, 200, 100, 50, and 20 µg/ml.

2.4. Cytotoxicity Testing Using the MTT Assay

The MTT assay is conducted to measure cell growth and evaluate metabolic activity, serving as a key method for assessing cytotoxicity. The test is typically performed in darkness due to the light sensitivity of the MTT reagent [10].

3. Results and discussion

the MTT used cytotoxicity assay to investigate the toxic effect (BS) of the extract from B. bifidum bacteria on the colorectal cancer cell line HRT-18 and normal HdFn cells. This assay relies on the conversion of the yellow MTT compound into insoluble purple formazan through the action of the enzyme succinate dehydrogenase (found in mitochondria). The test results showed that (BS) demonstrated good efficacy against the HRT-18 cell line, inhibiting the cells by (68.4, 61.3, 48.3, 36, 24.4)% at concentrations of (400, 200, 100, 50, 25) µg/mL, respectively. Meanwhile, (BS) exhibited no acute cytotoxicity on normal HdFn cells, with inhibition percentages ranging from (19.6, 12.7, 6.7, 5.6, 4.7)% at the same concentrations, as shown in table (3-1). Moreover, the results showed significant differences ($P \leq 0.0001$) in the calculation of the concentration (IC50) when treating the cancerous HRT-18 cells with the biosurfactant (BS), which reached 53.4 µg/mL, compared to the normal HdFn cell line, which reached 218.2 µg/ml , as shown in Figure (3-1).

Table (3-1): The effect of the (BS) extracted from *B. bifidum* on the cell line HRT-18 and the normal cell line HdFn using the MTT assay after 24 hours of exposure at a temperature of 37°C.

(BS)				
Concen. µg mL^{-1}	Inhibition % (Mean±SD)		*p* value	LSD (t Value)
	HdFn	HRT-18		
400	19.56±0.75	68.44±1.34	≤ 0.0001 **	10.46
200	12.73±0.9	61.35±1.63	≤ 0.0001 **	
100	6.75±0.29	48.30±4.82	≤ 0.0001 **	
50	5.63±0.60	36.00±2.78	≤ 0.0001 **	
25	4.67±0.52	24.42±3.22	0.0005 **	
** *p* value ≤ 0.01				

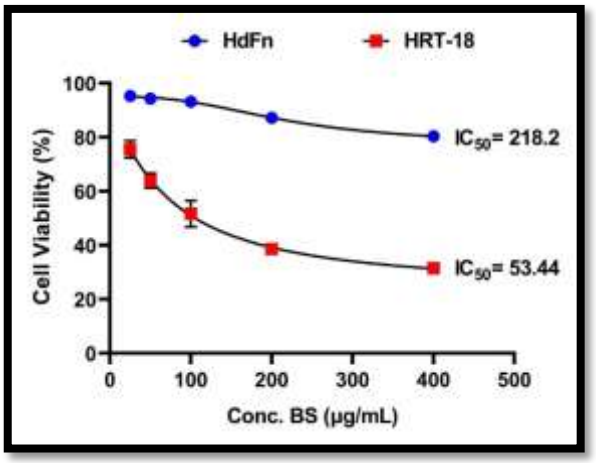

Figure (3-1) IC50 (mean±SD%) for cell line HRT-18 and normal cell line HdFn when treated (BS) extracted from B. bifidum using MTT test at 37°C, 5% CO2 for 24 hours.

Recent research suggests that probiotics can influence the growth of cancer cells and reduce their ability to spread. Although studies specifically focusing on the HRT-18 cell line are still limited, findings from other studies indicate that *B. bifidum* may have inhibitory effects on tumor growth [11]. Several studies have highlighted the potential effects of the probiotic *B. bifidum*, indicating that certain strains of *B. bifidum* can enhance the body's immune response, which may help reduce tumors. For instance, these strains have been shown to promote the production of Interferon-γ, a crucial molecule in the immune response [12].

The test results demonstrated that treating the colorectal cancer cell line HRT-18 and the normal cell line HdFn with the chemotherapy FOLFOX at various concentrations resulted in inhibition. The inhibition percentages for the HRT-18 cell line were (56.7, 48.8, 33.6, 19.6, 6.3)%, while for the normal cell line, they were (28, 17, 6.9, 5.9, 3.2)% at concentrations of (400, 200, 100, 50, 25) µg/mL, respectively, as shown in Table (3-2). The calculated IC50 values were 77.1 µg/mL for the cancerous HRT-18 cells and 174.6 µg/mL for the normal HdFn cell line, as shown in Figure (3-2).

Table (3-2) Effect of FOLFOX on HRT-18 cell line and HdFn cell line using MTT test at 37°C, 5% CO for 24 hours

FOLFOX				
Concen. µg mL^{-1}	Inhibition % (Mean±SD)		*p* value	LSD (t Value)
	HdFn	HRT-18		
400	28.05±3.05	56.76±2.74	0.0003 **	6.051
200	17.09±0.57	48.81±2.59	≤0.0001 **	
100	6.91±3.74	33.65±3.48	0.0008 **	
50	5.94±1.38	19.65±1.64	0.0004 **	
25	3.20±0.94	6.38±4.04	0.2553 NS	
p* value ≤ 0.01 , NS: Non-significant*				

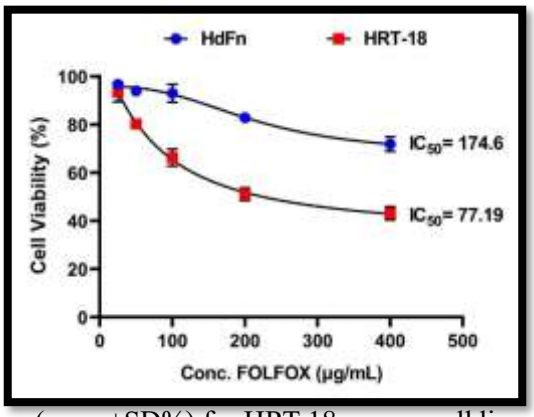

Figure (3-2) IC50 curve (mean±SD%) for HRT-18 cancer cell line and HdFn normal cell line with FOLFOX chemotherapy using MTT test at 37°C, 5% CO2 for 24 hours

FOLFOX is a combination chemotherapy regimen consisting of (5-fluorouracil, folinic acid, and oxaliplatin) , used for treating colorectal cancer. In this context researchers have studied the effect of FOLFOX on cancerous and normal cell lines under specific conditions using the MTT assay to evaluate its impact [13]. It can have a significant inhibitory effect on the growth of colorectal

cancer cells, and the results indicated a substantial reduction in cell viability following exposure [14]. The findings also showed that FOLFOX affects normal cell lines; however, the response may vary depending on the biological characteristics of each cell line Importantly , this treatment may cause cytotoxicity, impacting healthy cells, which necessitates the development of strategies to mitigate these adverse effects [15].

The results showed significant differences ($P \leq 0.0001$) between the (BS) and the FOLFOX in the cancerous cell line HRT-18 at concentrations of (400, 200, 100, 50, 25) µg/mL, as illustrated in Figure (3-3). Meanwhile the results indicated significant differences between the (BS) and the FOLFOX in the normal cell line HdFn at concentrations of (400, 200) µg/mL, but no significant differences were observed at concentrations of (100, 50, 25) µg/mL, as shown in Figure (3-4).

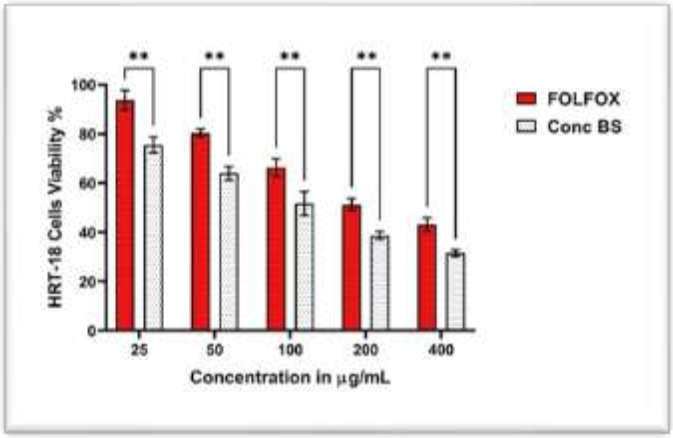

Figure (3-3) Comparison of the effect of BS and FOLFOX on the HRT-18 cell line using the MTT test at 37°C, 5% CO for 24 hours

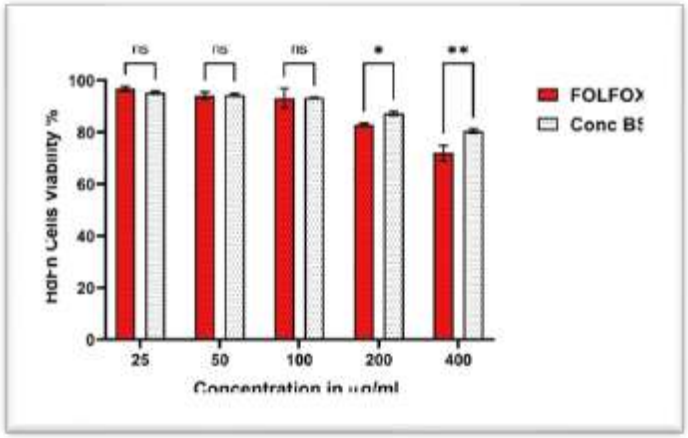

Figure (3-4) Comparison of the effect of BS and FOLFOX on the normal HdFn cell line using the MTT test at 37°C, 5% CO for 24 hours

It is believed that FOLFOX inhibits DNA synthesis, through the interaction of oxaliplatin with cellular DNA, which triggers a cellular response that leads to apoptosis [14]. Clinical studies have proven its effectiveness in improving survival rates, but the development of treatment resistance calls for the search for new strategies to enhance the effectiveness of FOLFOX [17].Clinical studies have reported the development of resistance in colorectal cancer cells, including the HRT-18 cell line, to chemotherapy such as FOLFOX. This resistance is often associated with increased phosphorylation in signaling pathways such as AKT/mTOR , leading to negative therapeutic outcomes , The results may vary depending on the level of resistance to treatment in different cell lines. [16]. The current study indicated that FOLFOX exhibits inhibitory effects against the HRT-18 and HdFn cell lines, with differences in response between the two lines. Meanwhile, BS demonstrated similar inhibitory effects on the cancerous HRT-18 cell line , but with significantly lower cytotoxic effects on the normal HdFn cell line. The use of the MTT assay is considered an effective tool for evaluating these effects, providing valuable insights for developing new therapeutic strategies.

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