Research Article:
The Probiotic Bacteria Induce Apoptosis in Breast and Colon Cancer Cells: An Immunostimulatory Effect

Zahra Khosrovan[1], Setareh Haghighat[2], Mehdi Mahdavi[3,4*]

1. Department of Microbiology, Faculty of Advanced Technologies, Tehran Medical Sciences Branch, Islamic Azad University, Tehran, Iran.
2. Recombinant Vaccine Research Center, Tehran University of Medical Sciences, Tehran, Iran.
3. Department of Immunotherapy, Institute of Pharmaceutical Sciences, Tehran University of Medical Sciences, Tehran, Iran.
4. Department of Immunology, Pasteur Institute of Iran, Tehran, Iran.

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ABSTRACT

Background: Uncontrolled cell proliferation and resistance to apoptosis are the main characteristics of cancer cells. Therefore, a substance with the capability to induce apoptosis in cancer cells could be known as an anti-cancer material. Probiotics are useful microorganisms that are crucial for the host's health.

Materials and Methods: In the present study, we evaluated the synergic effect of probiotic bacteria on the cytotoxic potency of cancer cells and apoptosis genes expression. Lactobacillus acidophilus and Bifidobacterium bifidum were cultured in MRS broth and then MTT test was performed on AGS, MCF-7, and peripheral mononuclear cells after treatment with the bacterial corpse. β-actin, Bcl2, Bax, TNF-α, and IFN-γ gene expression were evaluated in treated cell lines by real-time PCR method.

Results: The result of cytotoxicity assay showed that bacterial corpse has higher cytotoxicity effects on cancer cells compared with normal cells. Results of gene expression demonstrated that Bcl2 and Bax gene expression significantly increased in cancer cells and TNF-α gene expression remarkably increased in normal cells as compared with the control group.

Conclusion: Probiotic bacterial corpse can induce apoptosis in cancer cells. In normal cells, the immune system shifts responses toward Th1 and inflammatory cytokines.

Introduction

Probiotics are live and beneficial microorganisms that affect microbial flora and keep the host in healthy condition. Probiotics belong to a large group of key bacteria of intestine microbial flora in humans where they live without causing harm to the host [1].

Probiotic bacteria are intestine natural flora which could help digestion of complex molecules and produce vitamins and different antibiotics that are useful for

*** Corresponding Author:**
Mehdi Mahdavi, PhD.
Address: *Recombinant Vaccine Research Center, Tehran University of Medical Sciences, Tehran, Iran.*
Phone: *+98 (21) 66909128*
E-mail: *m_mahdavi@pasteur.ac.ir; mahdavivac@gmail.com*

health. These bacteria have a beneficial effect on some diseases such as diarrhea in children. Also, maintaining homeostasis in the performance of acid lactic bacteria is another probiotic effect. They can facilitate lactose digestion and help the immune responses against infections and also prevent colon cancer growth, genitourinary system infectious, and treat food allergies [2].

Probiotics have beneficial effects for the host such as the production of preventive compounds and inhibiting competition against pathogenic agents for chemical materials and stimulating connection places and regulate immune system activity and improve intestine microbial balance [3, 4]. Moreover, probiotics are known not only as growth inducer but also as the immune system stimulator and prevent many infectious diseases [5]. Today, Lactobacillus and Bifidobacterium are often used as probiotics and also most of them are recognized harmless [6].

The Lactobacillus bacteria have the basil and coco-basil shape; they are gram-positive, non-spore, and catalase, oxidase, and indole negative. They belong to the Lactobacillacea family. Lactobacillus strains potentiate the intestine mucosal barrier which could keep and promote the immune status and decrease bacteria movement. Also, they decrease intestinal inflammatory diseases and inflammatory bowel syndrome. Cancer common therapeutics such as surgery, radiotherapy, and chemotherapy could kill the tumor and also normal cells. Therefore, complications and adverse effects are seen in these patients [7].

Acid lactic bacteria can prevent colon cancer with certain mechanisms such as alteration of metabolic activities of intestine micro-flora, alteration of colon chemical and physical situation, binding to carcinogens and destroying them, quantity and quality changes of intestine micro-flora and inhibition of carcinogenic compounds such as ammonia, secondary bile acids, production of anticancer substances, and boosting host immunity [8].

Some studies demonstrated that probiotics intake could increase Natural Killer (NK) cells activity and immunoglobulin status. Moreover, probiotics have anticancer performance through apoptosis induction. Probiotic bacteria can be considered as a therapeutic agent with high safety and without any side effects [9]. Probiotics can reduce fecal enzyme activity, prevent intestine harmful bacteria, and decrease soluble bile acid. In addition, secreted metabolites by probiotics could affect epithelial cells which result in decreased proliferation of cancerous cells [10]. Several studies showed that the simultaneous use of two probiotics could have synergistic effects. For the first time in the present study, we evaluated the synergistic effect of Lactobacillus acidophilus and Bifidobacterium bifidum on the immune system and the cytotoxic effects on cancerous cells and gene profiles which were involved in apoptosis and stimulating the immune system.

Materials and Methods

Probiotic bacteria culture

A standard strain of Lactobacillus acidophilus (PTCC:1643) and Bifidobacterium bifidum (PTCC:1644) were prepared from the microbial collection of Scientific Industrial Research Organization of Iran. Initially, lyophilized cells were cultured in MRS broth for 24 h. After centrifuging, the supernatant was discarded and the residual bacteria was frozen at -70° C and refrozen at 50° C for 5 times. Finally, the bacterial cells were sonicated and bacteria pellet was provided for cytotoxicity assay with various concentrations of the cells.

Cell lines culture

In the present study, MCF-7 (NCBI No: C135) and HT-29 (NCBI No: C466) cell lines were utilized and provided from Pasture Institute in Tehran. The cellular suspension was transferred into DMEM medium containing 10% FBS and some antibiotics such as streptomycin (100 µg/mL), penicillin (1000 U/mL).

Cytotoxicity assay

The MTT assay was performed to evaluate the cytotoxicity effect of the extraction of Lactobacillus acidophilus and Bifidobacterium bifidum on HT-29 and MCF7 cell lines. First, 100 µL suspension of bacteria was cultured in RPMI-1640 medium containing 10% FBS serum. Then, the cells were incubated at 37° C and 5% CO2 for 24 h. Diluted extracts with various concentrations of each extraction were added to segregated wells in triplicate. After incubation for 24, 48, and 72 h, 20 µL of MTT solution was dispensed to each well and incubated for 4 h again. Finally, the supernatant was discarded and 100 µL of DMSO added to each well, and the plates were read by ELISA reader in 570 wavelengths.

Gene expression assay

Cellular RNA was extracted by total RNX plus kit (CinnaGen, Iran) based on the manufacturer's instructions. Then, cDNA was synthesized by using the cDNA synthesis kit (Yekta-Tajhiz, Iran) according to the manufacturer's instructions. In the present study, Bcl2 and Bax gene expression was evaluated in cancerous cells,

and also IFN-γ gene expression in normal cells was measured by real-time PCR. Also, Bcl2 and Bax gene expression in treated HT-29 cell line were evaluated. In addition to primers of Bcl2 and Bax genes, one more pair of primers was designed for the β-actin gene as a housekeeping gene. In addition, the master mix (Bioneer), primers of β-actin, Bcl2, and Bax genes were used duplicate, and also cDNA IFN-γ gene expression was measured in normal cells. Finally, the quality and quantity of extracted RNAs were evaluated by NanoDrop and electrophoresis machine.

Statistical analysis

Data analysis was carried out by GraphPad Prism and the results were evaluated by 1-way ANOVA test. Also, target genes expression between control and treated samples was measured by the Tukey HSD post hoc test. Data analysis of real-time PCR was performed by threshold cycle and its formula is $2^{-\Delta\Delta Ct}$.

Results

Cytotoxic assay

HT-29 cell line

The treated cell line with Lactobacillus acidophilus extract after 24 h incubation demonstrated that dilutions of 1:4 up to 1:32 remarkably increase the inhibition of cell growth between the treated cells group and untreated cell line as the negative control group (P<0.0001). Also regarding the inhibition of the cell growth, the result showed no significant difference in the treated cells as compared with the untreated cell line as the negative control group at a dilution of 1:2. In this regard, a treated cell line with Bifidobacterium bifidum after 24 h incubation demonstrated a significant increase in the inhibition of cell growth as compared with the untreated cell line as a negative control group at dilutions of 1:4 up to 1:16 (P<0.0001). There was no significant increase in the treated cells versus the untreated cell line as the negative control group in dilutions of 1:2 and 1:32. In other assessment after 24 h incubation, the results of treated cells with mixture extracts of Lactobacillus acidophilus and Bifidobacterium bifidum significantly increased the inhibition of cell growth compared with the untreated cell line as the negative control group in dilutions of 1:4 up to 1:32 (P<0.0001) (Figure 1).

Treatment of HT-29 cell line with Lactobacillus acidophilus extract after 48 h significantly increased the inhibition of cell growth versus the untreated cell line as the negative control group in dilutions of 1:2 up to 1:32 (P<0.0001). Also, the treated cell line with Bifidobacterium bifidum extract increased remarkably the inhibition of cell growth in dilutions of 1:2 up to 1:8 versus the untreated cell line as the negative control group (P<0.0001). In dilutions of 1:16 and 1:32, there was no remarkable difference in the treated cell line with Bifidobacterium bifidum extract as compared with the untreated cell line as the negative control group. Also, the obtained results from a treated cell line with mixture extracts of lactobacillus acidophilus and Bifidobacterium bifidum bacteria showed no significant difference from the untreated cell line as the negative control group in dilutions of 1:2 up to 1:32 after 48 h treatment (Figure 2).

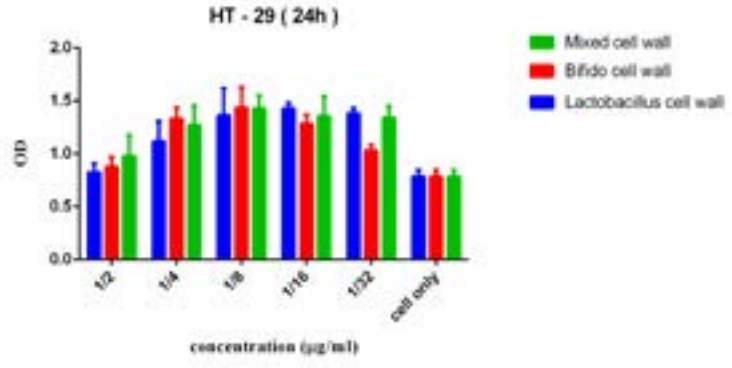

Figure 1. Cytotoxicity assay after 24 h

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The cytotoxicity effect of Lactobacillus acidophilus and Bifidobacterium bifidum and their mixture was evaluated on the HT-29 cell line in different dilutions by the MTT method Lactobacillus acidophilus, Bifidobacterium cell wall, and their mixture showed an increasing gradient of cytotoxicity (in dilutions of 1:4 up to 1:8) after 24 h incubation. The synergistic effect of acidophilus and bifidum is observed in the inhibition of growth cells compared with the untreated cells. Both bacteria and their combination showed the cytotoxicity effect on colon carcinoma cell line (P<0.05, as a significant statistical value).

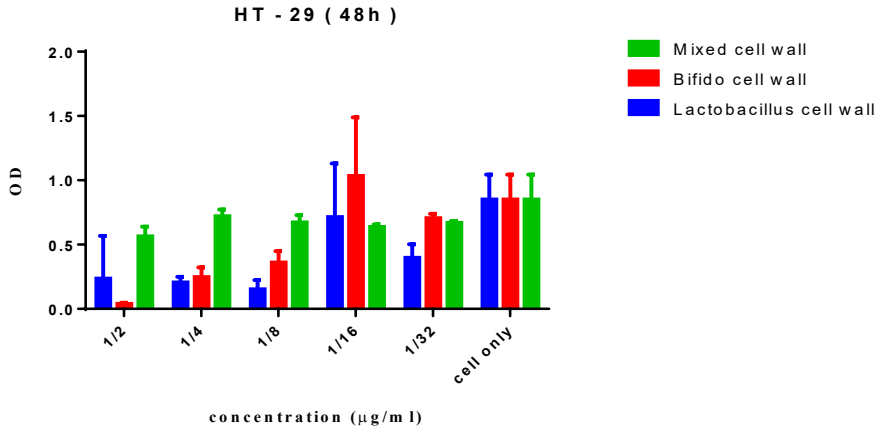

Figure 2. Cytotoxicity assay after 48 h

The cytotoxicity effect of Lactobacillus acidophilus and Bifidobacterium bifidum and their mixture was evaluated on the HT-29 cell line in different dilutions by the MTT method. Lactobacillus acidophilus and Bifidobacterium have a cytotoxicity effect on HT-29 colorectal carcinoma cell (1:2 to 1:8). There is no significant difference between the treated and untreated groups in the presence of a mixture of both bacterial cell walls (dilutions of 1:2 up to 1:32) ($P<0.05$, as a significant statistical value).

After 72 h incubation and in dilutions of 1:2 up to 1:32, the results did not show any significant difference in the treatment of HT-29 cell line with Lactobacillus acidophilus extract as compared with the untreated cell line as the negative control group. Also, in dilution of 1:2, the inhibition of cell growth was significantly increased in the treatment cell line with Bifidobacterium bifidum extract compared with the untreated cell line as the negative control group ($P<0.0296$). However, there was no significant difference in the treated group versus the untreated cell line as the negative control group in dilutions of 1:4 up to 1:32. After 72 h incubation, the results of the treatment of HT-29 cell line with mixture extracts of Lactobacillus acidophilus and Bifidobacterium bifidum did not show a remarkable difference in comparison to the untreated cell line as the negative control group in dilutions of 1:2 up to 1:32 (Figure 3).

MCF-7 cell line

After 24 h incubation, the treated MCF-7 cell line with Lactobacillus acidophilus extract significantly increased the inhibition of cell growth versus the untreated cell line as the negative control group in dilutions of 1:2 and 1:4 ($P<0.0001$). However, there was no significant difference between the treated cells with Lactobacillus acidophilus

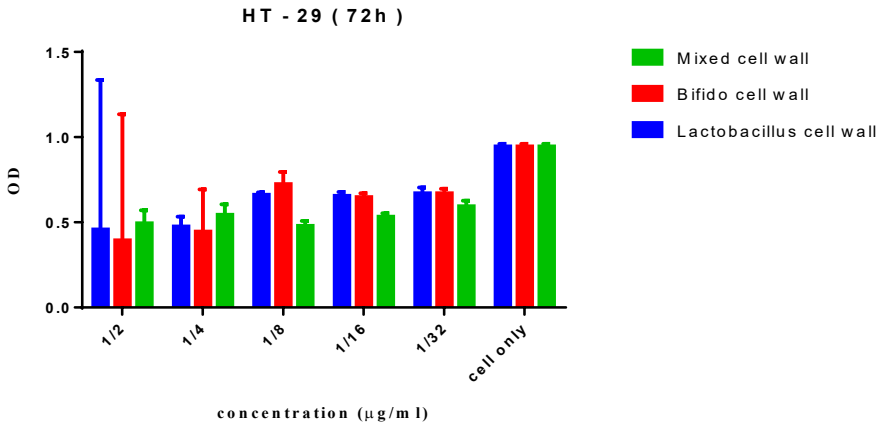

Figure 3. Cytotoxicity assay after 72 h. The cytotoxicity effect of Lactobacillus acidophilus and Bifidobacterium bifidum and their mixture was evaluated on the HT-29 cell line in different dilutions by the MTT method. The cytotoxicity effect of Bifidobacterium bifidum on HT-29 was observed in 1:2 dilution. Also, there were no significant differences between other experimental groups in other dilutions versus each other ($P<0.05$, as a significant statistical value).

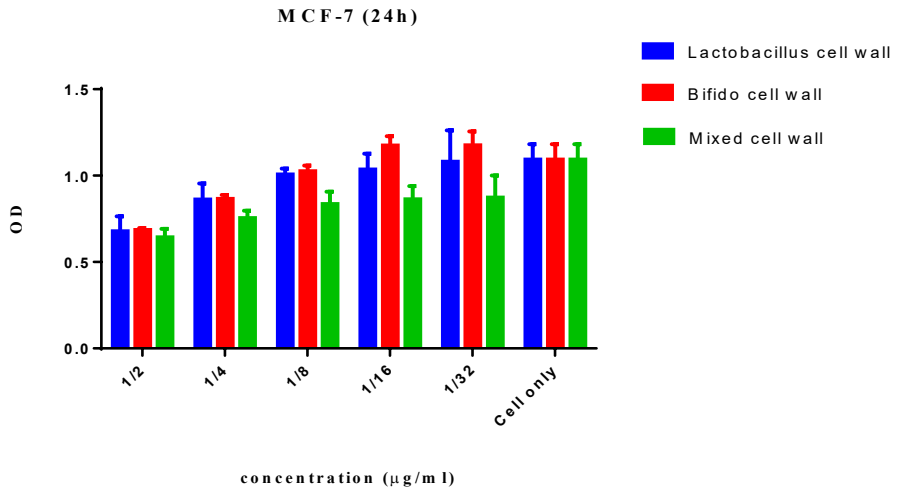

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Figure 4. Cytotoxicity assay after 24 h

The cytotoxicity effect of Lactobacillus acidophilus and Bifidobacterium bifidum and their mixture was evaluated on the MCF7 cell line in different dilutions by the MTT method. The significant cytotoxicity effect of both bacterial cell walls on the MCF-7 cell line was observed. The combination of these bacteria showed remarkable cytotoxicity effects on the MCF-7 breast cancer cells in dilutions of 1:2 up to 1:32 ($P<0.05$, as a significant statistical value).

extract and the untreated cell line as the negative control group in dilutions of 1:8 up to 1:32. Also in treatment with Bifidobacterium bifidum extract, the elevation of cell growth inhibition was observed in dilutions of 1:2 and 1:4 in comparison to the untreated negative cell line as the control group ($P<0.0001$).

But, there was no significant difference in the treated cells with Bifidobacterium bifidum extract versus the untreated cell line as the control group in dilutions of 1:8 up to 1:32. There was a remarkable increase in the inhibition of cell growth through the treatment of MCF-7 cell line with mixture extracts of Lactobacillus acidophilus and Bifidobacterium bifidum in comparison to the untreated cell line as the negative control group in dilutions of 1:2 up to 1:32 ($P<0.0001$) (Figure 4).

Results of cytotoxicity assay after 48 h incubation time showed that the treated cells with Lactobacillus acidophilus extract increase remarkably the inhibition of cell growth versus the untreated cell line as the negative control group in dilutions of 1:2 up to 1:16 ($P<0.0001$).

Treatment of MCF-7 cell line with Bifidobacterium bifidum extract remarkably increased the inhibition of cell growth in comparison to the cell line as the negative

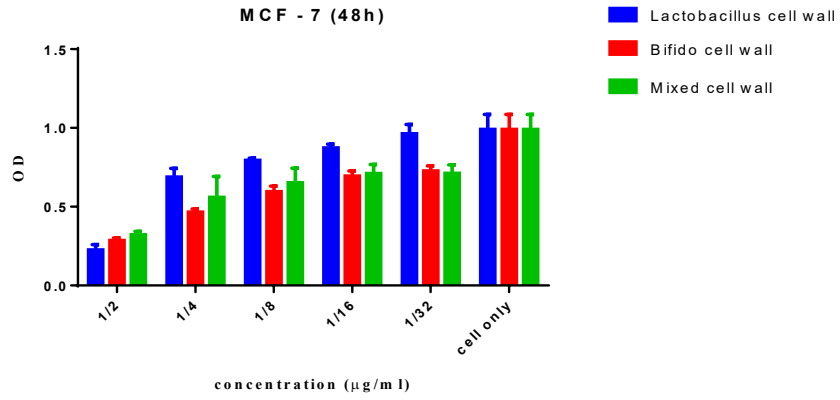

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Figure 5. Cytotoxicity assay after 48 h

The cytotoxicity effect of Lactobacillus acidophilus and Bifidobacterium bifidum was evaluated on the MCF7 cell line in different dilutions by the MTT method. The cytotoxicity effects of these bacterial cell wall and their combination on the MCF-7 cell line were observed in most dilutions ($P<0.05$, as a significant statistical value).

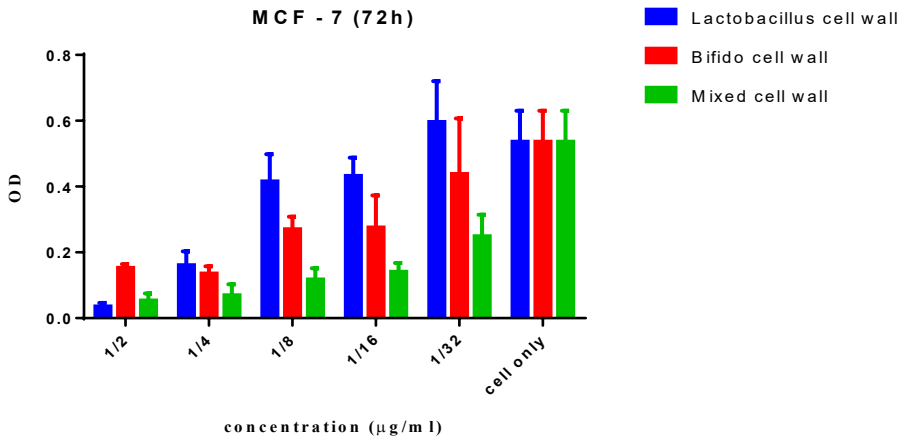

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Figure 6. Cytotoxicity assay after 72 h

The cytotoxicity effect of Lactobacillus acidophilus and Bifidobacterium bifidum and their mixture was evaluated on the MCF7 cell line in different dilutions by the MTT method. The cytotoxicity effects of these bacterial cell wall on MCF-7 were observed in some dilutions. Also, the mixture of both probiotic bacteria showed cytotoxicity effects in all concentrations (P<0.05, as a significant statistical value).

control group in dilutions of 1:2 up to 1:32 (P<0.0001). Inhibition of cell growth significantly increased after treatment with the mixture extracts of Lactobacillus acidophilus and Bifidobacterium bifidum in comparison to the untreated cell line as the negative control group in dilutions of 1:2 up to 1:32 (P<0.0001) (Figure 5).

After 72 h incubation, the treatment of MCF-7 cell line with Lactobacillus acidophilus extract demonstrated a significant increase compared with the untreated cell line as the negative control group in dilutions of 1:2 and 1:4 (P<0.0001). Also, in dilutions of 1:2 up to 1:16, the results showed a remarkable increase in the treated cell with Bifidobacterium bifidum extract as compared with the untreated cell line as the negative control group (P<0.0001). Inhibition of cell growth increased in treatment with mixture extracts of Lactobacillus acidophilus and Bifidobacterium bifidum compared with the untreated cell line as the negative control group in dilutions of 1:2 up to 1:32 (P<0.0001) (Figure 6).

Cytotoxicity assay on peripheral blood mononuclear cells (PBMC)

After 24 h incubation, analysis of the results demonstrated that the treated normal cells with lactobacillus acidophilus extract remarkably decreased as compared with the untreated cell line as the negative control group in dilutions of 1:2 up to 1:32 (P<0.0001). Also, normal cells treated with Bifidobacterium bifidum showed a

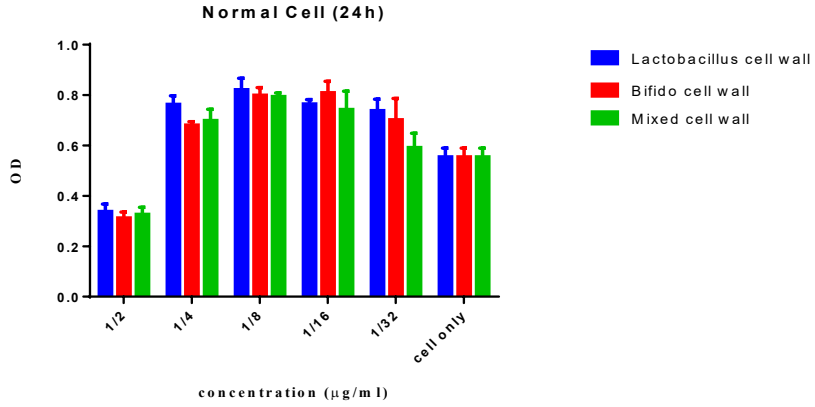

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Figure 7. Cytotoxicity assay after 24 h

The cytotoxicity effect of Lactobacillus acidophilus and Bifidobacterium bifidum and their mixture on PBMCs was evaluated by the MTT method in different dilutions. The result showed significant cytotoxicity on normal cells treated with these bacteria and their mixture versus untreated cells (P<0.05, as a significant statistical value).

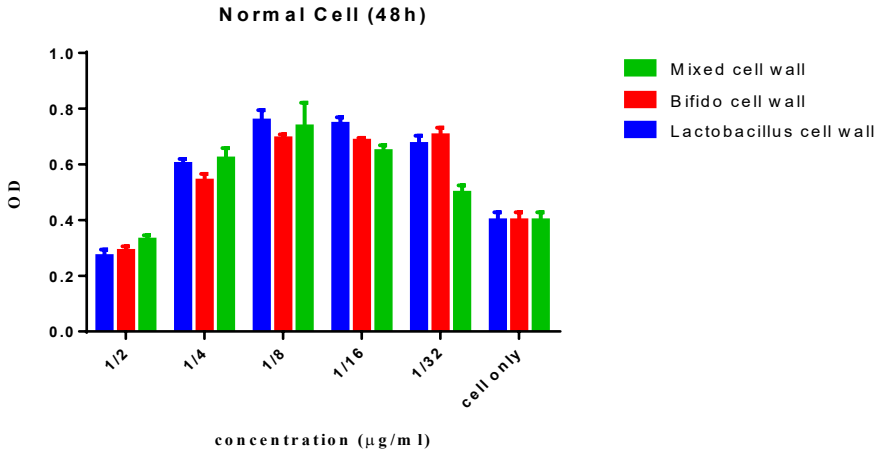

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Figure 8. Cytotoxicity assay after 48 h

The cytotoxicity effect of Lactobacillus acidophilus and Bifidobacterium bifidum and their mixture on PBMCs was evaluated by the MTT method in different dilutions. There were no significant cytotoxicity effects on the normal cells treated with the cell wall of bacteria and their mixture versus the untreated cells (P<0.05, as a significant statistical value).

significant increase as compared with the untreated cell line as the negative control group in dilutions of 1:2 up to 1:32 (P<0.0001). Normal cells treated with mixture extracts of lactobacillus acidophilus and Bifidobacterium bifidum remarkably increased the inhibition of cell growth versus the untreated cell line as the negative control group (P<0.0001) (Figure 7).

After 48 h incubation, assessment of the results demonstrated that treated normal cells with lactobacillus acidophilus extract did not show any significant differences from the untreated cell as the negative control group in dilutions of 1:2 up to 1:32. Also, normal cells treated with Bifidobacterium bifidum did not show any significant differences compared with the untreated cells as the negative control group in dilutions of 1:2 up to 1:32. Normal cells treated with the mixture of Lactobacillus acidophilus and Bifidobacterium bifidum extracts did not show any remarkable difference in inhibition cell growth as compared with the untreated cells as the negative control group (Figure 8).

After 72 h incubation, the results of treatment with Lactobacillus acidophilus extract demonstrated a remarkable increase in comparison to the untreated cell as the negative control group in dilutions of 1:2 up to 1:32 (P<0.0001). Also in dilutions of 1:2 up to 1:16, the results showed a significant increase in the treated cells with Bifidobacterium bifidum extract versus the untreated cells as the negative control group (P<0.0001).

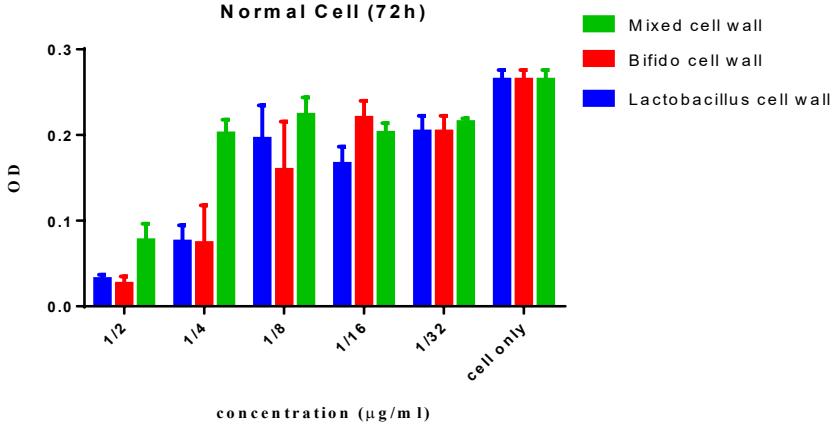

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Figure 9. Cytotoxicity assay after 72 h

The cytotoxicity effect of Lactobacillus acidophilus and Bifidobacterium bifidum and their mixture on PBMCs was evaluated by the MTT method in different dilutions. The cytotoxicity effect of the cell wall of both bacteria and their mixture were significant in the inhibition of cell growth in the treated cells compared with the untreated cells (P<0.05, as a significant statistical value).

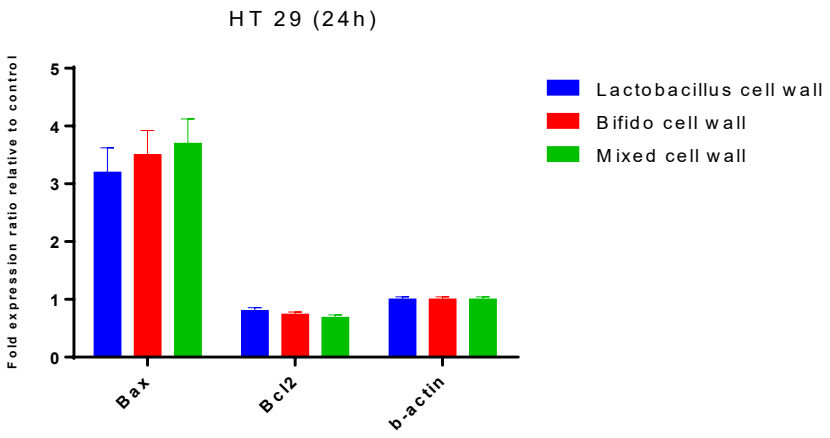

Figure 10. Apoptotic gene expression after 24 h

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The expression of Bax, Bcl2, and β-actin genes after treatment with Lactobacillus acidophilus and Bifidobacterium bifidum and their mixture on HT-29 was measured by real-time PCR. The mRNA level of Bax was significantly upregulated in the treated cells with these bacteria and their mixture versus the reference gene.

The inhibition of cell growth was increased by the treatment with the mixture of Lactobacillus acidophilus and Bifidobacterium bifidum extracts compared with the untreated cells as the negative control group in dilutions of 1:2 up to 1:16 ($P<0.0001$) (Figure 9).

Apoptotic gene expression

Results of gene expression assay on the HT-29 cell line after 24, 48, and 72 h showed that the effect of Lactobacillus on the expression of some genes is remarkable. There was a significant increase in gene expression of Bax after 24, 48, and 72 h ($P<0.0001$). Effect of Bifidobacterium cell wall on Bax and BCL2 was evaluated. Results showed a significant increase in gene expression of Bax after 24, 48, and 72 h incubation ($P<0.0001$). Also, the effect of the Lactobacillus cell wall when mixed with the Bifidobacterium cell wall was investigated. The results demonstrated a remarkable reduction in the gene expression of Bax after 24, 48, and 72 h incubation ($P<0.0001$). But, other results did not show any significant difference between the comparable groups (Figures 10, 11, and 12).

Additionally, the gene expression of Bax and Bcl2 on the MCF-7 cell line was evaluated by real-time PCR method at 24, 48, and 72 h after incubation. Results showed that the effect of the Lactobacillus cell wall significantly increased Bax gene expression after 24, 48, and 72 h incubation. Also, there was a remarkable de-

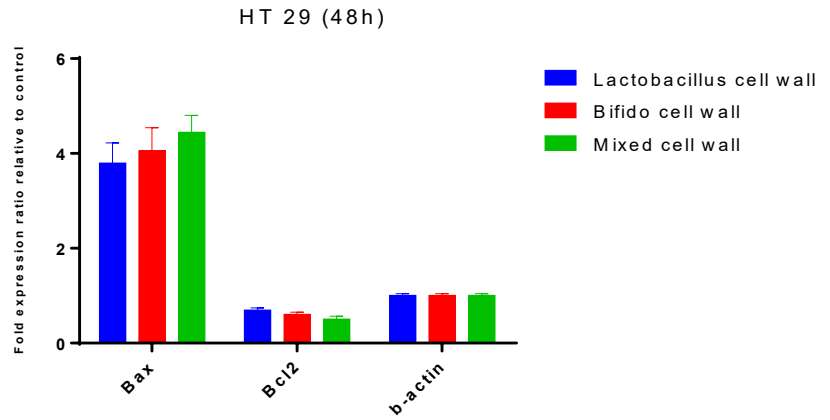

Figure 11. Apoptotic gene expression after 48 h.

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The expression of Bax, Bcl2, and β-actin genes after treatment with Lactobacillus acidophilus and Bifidobacterium bifidum and their mixture on HT-29 was measured by real-time PCR. The gene expression of Bax was significantly increased in the treated cells with these bacteria and their mixture versus the reference gene.

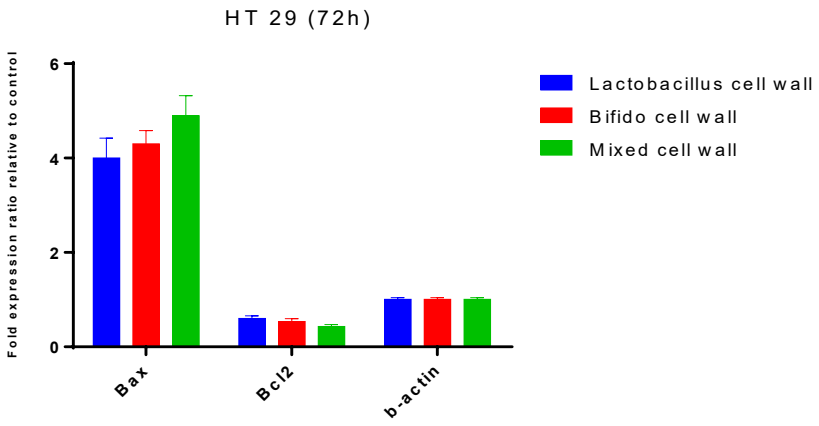

Figure 12. Apoptotic gene expression after 72 h.

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The expression of Bax, Bcl2, and β-actin genes after treatment with Lactobacillus acidophilus and Bifidobacterium bifidum and their mixture on the HT-29 cell line was measured by real-time PCR. The mRNA level of Bax was significantly upregulated in the treated cells with these bacteria and their mixture versus the reference gene.

crease in Bax gene expression when it was treated with Bifidobacterium cell wall after 24, 48, and 72 h incubation. Results of the mixture effect of Lactobacillus and Bifidobacterium cell wall showed a significant increase in Bax gene expression after 24, 48, and 72 h incubation (Figures 13, 14, and 15).

IFN-γ and TNF-α gene expression in PBMCs

After 72 h treatment, the results of IFN-γ and TNF-α gene expression assessment in PBMCs treated with two probiotic bacteria and their mixture demonstrated a non-significant increase in IFN-γ gene expression. However, there was a significant increase in TNF-α gene expression ($P<0.0128$) (Figure 16).

Discussion

Today, cancer is considered as a serious problem in the human community. An increase in the number of cancer patients has raised concerns in this respect and more efforts are needed to find novel therapeutic approaches. One type of cancer is breast cancer with 694 deaths reported in 2017 in women [11]. Generally, chemotherapy, radiotherapy, and surgery are the common therapeutic ways, but they could damage the normal cells along with the tumor cells. Thus, various research studies have been performed in this field. Boosting adaptive immunity as an important target could be considered to remove the tumor cells by the specific attack of adaptive immune responses [12].

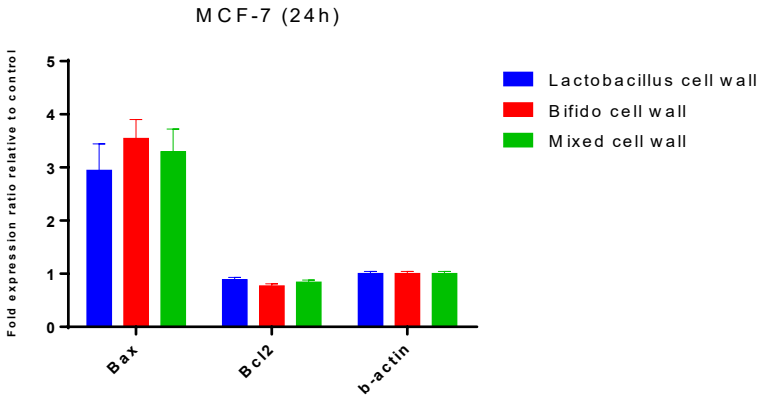

Figure 13. Apoptotic gene expression after 72 h.

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The expression of Bax, Bcl2, and β-actin genes after treatment with Lactobacillus acidophilus and Bifidobacterium bifidum and their mixture on the HT-29 cell line was measured by real-time PCR. The mRNA level of Bax was significantly upregulated in the treated cells with these bacteria and their mixture versus the reference gene.

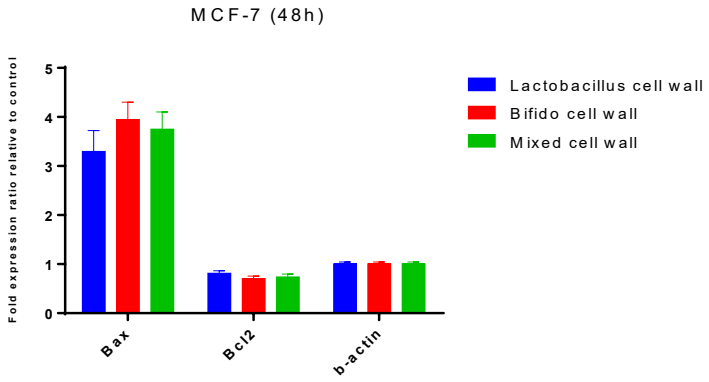

Figure 14. Apoptotic gene expression after 48 h

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The expression of Bax, Bcl2, and β-actin genes after treatment with Lactobacillus acidophilus and Bifidobacterium bifidum and their mixture on MCF-7 was measured by real-time PCR. The mRNA level of Bax was significantly upregulated in treated cells with these bacteria and their mixture versus the reference gene.

On the other hand, probiotics have been introduced as beneficial bacteria for the digestive system. Lactobacillus and Bifidobacterium are probiotic bacteria that could reinforce the immune system. Moreover, probiotic bacteria can shift Th1 immune responses in infectious and cancer models. Also, the function of innate immune cells, including macrophages, dendritic cells, and natural killer cells is potentiated through these probiotic bacteria which in turn could stimulate adaptive immunity and consequently inhibition of the tumor growth [13, 14]. In the present study, anticancer properties of Lactobacillus acidophilus and Bifidobacterium bifidum as two probiotic bacteria were evaluated on HT-29 and MCF7 cancer cell lines. In the present study, the best tumoricidal effect of Lactobacillus acidophilus and Bifidobacterium bifidum on HT-29 and MCF7 cancer cell lines was achieved at 24, 48, and 72 h of incubation.

Mahmudi et al. studied the effect of Bifidobacterium bifidum on Caco II cancer cell line and showed that inhibitory effect of Bifidobacterium bifidum supernatant on cancer cells was 55% to 82% related to 100 µL/mL and 300 µL/mL concentration for 24 and 72 h treatment, respectively [15]. Also in another study, Dallal et al. got the same results as ours [16]. Our results revealed that the effects of probiotics are different in comparison to each other and based on tumor cell type. Several studies have approved the antitumor effect of Lactobacillus acidophilus and Bifidobacterium bifidum [17, 18]. This experiment demonstrated that the mixture of Lactobacillus acidophilus and Bifidobacterium bifidum have a boosting effect in tumor inhibition of the MCF7 cell line. Ghezelbash et al. showed that probiotic subscriptions decreased significantly the tumor growth as compared with the control group. Potentiat-

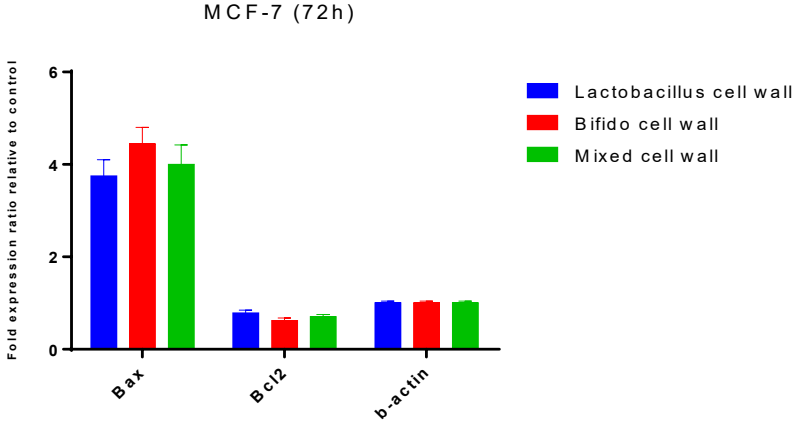

Figure 15. Apoptotic gene expression after 72 h

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The expression of Bax, Bcl2, and β-actin gene after treatment with Lactobacillus acidophilus and Bifidobacterium bifidum and their mixture on MCF-7 was measured by real-time PCR. The mRNA level of Bax was significantly upregulated in the treated cells with these bacteria and their mixture versus the reference gene.

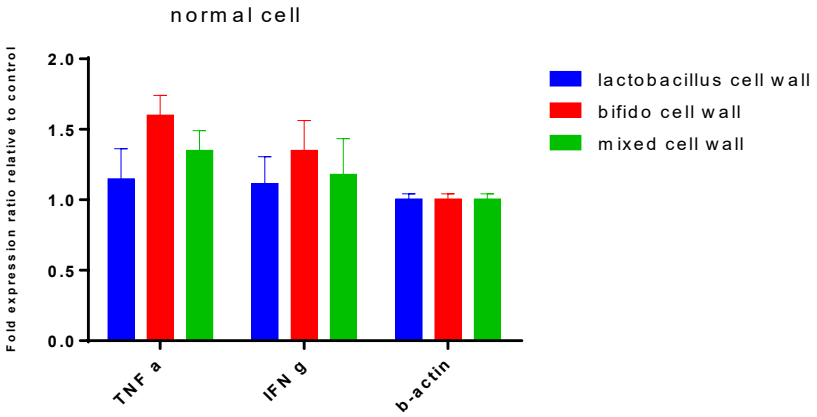

Figure 16. IFN-γ, TNF-α, and β-actin gene expression after 72 h

IMMUNOREGULATION

The expression of IFN-γ, TNF-α, and β-actin gene was evaluated after treatment with Lactobacillus acidophilus and Bifidobacterium bifidum and their mixture on PBMCs by real-time PCR. There was no significant difference between the IFN-γ expression versus the reference gene. But, the remarkable increase was observed in the gene expression of TNF-α versus the reference gene.

ing the immune system is a hallmark of anticancer properties in the Lactobacillus bacterium [19].

PBMCs cytotoxicity assay revealed that Lactobacillus acidophilus significantly decreases cell growth, but Bifidobacterium bifidum and the mixture of these two bacterial cell walls increase cell growth after 24 h treatment. Also after 48 h treatment with Lactobacillus acidophilus, Bifidobacterium bifidum, and their mixture, no significant differences were seen between the treated and untreated cells. Also, after 72 h treatment with Lactobacillus acidophilus or Bifidobacterium bifidum, cell growth increased significantly. However, treatment with their mixture could not show significant differences after 72 h treatment. Bax gene expression was increased by Lactobacillus acidophilus treatment. Also, Asghari et al. studied the cytotoxicity effect of Lactobacillus casei as a probiotic bacteria on HT-29 cancer cell line and evaluation of Bax and Bcl2 apoptotic genes expression. Their results demonstrated that Bax and Bcl2 gene expression increased after treatment with Lactobacillus casei [20].

Regarding the animal studies, researchers could confirm the hypothesis that immune responses could be increased following to the intake of cell wall of Lactobacillus acidophilus, Bifidobacterium bifidum and their mixture which in turn promotes antitumor activity against breast cancer [21]. In this regard, Dallal et al. showed that Lactobacillus acidophilus has this characteristic in the mice with breast cancer [16]. Baldwin et al. showed that Lactobacillus acidophilus and Lactobacillus casei could increase apoptosis induction in LS513 carcinoma cell line and the bacteria could be adopted as adjuvant or chemotherapy in therapeutic approaches [8].

Several studies demonstrated that these probiotic bacteria can increase IFN-γ, TNF-α, and type-I cytokines that agree with our scientific findings. This matter indicates that these studied probiotic bacteria may also suppress and remove the tumor through boosting the immune system [8, 22]. It seems that Lactobacillus acidophilus and Bifidobacterium bifidum have antitumor properties, yet their antitumor effect is various in each cell. On the other hand, it has a direct and or indirect antitumor effect on boosting the immune system by the production of IFN-γ, TNF-α cytokines thereby killing tumor cells. In this study, there were financial constraints to perform experiments in the animal model and evaluate protein concentrations or CD markers and other molecular assays.

Our findings indicate that \Lactobacillus acidophilus and Bifidobacterium bifidum have antitumor properties; however, this feature is different for each type of cell. Based on our results, these probiotic bacteria had more effect on breast cancer than colorectal cancer. In other words, their effectiveness on MCF7 is more than the HT-29 cell line. Also, the mixture of these bacteria could have a synergistic effect. This mixture of probiotic bacteria cell walls displayed an antitumor effect on the MCF7 but not the HT-29 cell line. Besides, these bacteria increase the apoptosis mechanism in tumor cells approved by our results based on Bax and Bcl2 gene expression. Finally, it seems that Lactobacillus acidophilus and Bifidobacterium bifidum with gene expression of inflammatory cytokines such as IFN-γ and TNF could potentiate

cellular immunity that is an indirect mechanism of anti-tumor probiotic bacteria.

Ethical Considerations

Compliance with ethical guidelines

All assays were performed according to the ethical principles of Helsinki. Also, because of in vitro study, there is no ethical guideline.

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Authors' contributions

Design: Mehdi Mahdavi; Implementation: Zahra Khosravan; Data analysis: Setareh Haghighat; Writting and revise the manuscript: All authors.

Conflicts of interest

The authors declared no conflict of interest.

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