

Research Article:

Secreted Chemicals From Probiotic Bacteria Potentiate Th1 Pattern of Immune Cells and Apoptosis Induction in Breast Cancer and Gastric Adenocarcinoma Cell Lines

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ABSTRACT

Cancer is one of the main causes of mortality. Therapeutic importance in some diseases, especially cancers has led to considering the anti-cancer effects of probiotics more than ever. Probiotics have stimulatory effects on the immunity and suppressor effects on cancer activity. Various studies report that probiotic bacteria have cytotoxicity effects on different types of solid tumors. In this research, we evaluated the synergic effect of secretory chemicals of *Lactobacillus* and *Bifidobacterium* bacteria on cytotoxic potency of breast and gastrointestinal cancer cell lines. *Bifidobacterium bifidum* and *Lactobacillus acidophilus* were cultured in MRS broth. Cytotoxicity assay was performed on AGS, MCF-7 cell lines, and peripheral mononuclear cells after treatment with bacteria extractions by MTT test. β -actin, *Bcl2*, *Bax*, *TNF- α* , and *IFN- γ* gene expression levels were evaluated in treated cell lines by real-time PCR method. The result of cytotoxicity assay showed that the obtained extraction from the bacterial cultures has a higher killing effect on cancer cells compared with normal cells. Results of gene expression indicated a significant increase in *Bax* and *Bcl2* gene expression in cancer cells. *TNF- α* gene expression increased significantly as compared with the control group. The obtained extraction from probiotics culture can induce death in cancer cells through the apoptosis mechanism and improve the cellular immune response, i.e., T helper 1 cytokine profile.

Introduction

E

xcessive cellular proliferation and resistance to apoptosis are key properties of cancer cells. So anticancer agents such as probi-

otic compounds that cause apoptosis in cancer cells could be utilized to treat cancer [1]. Cancer results from mutations in genes that control vital cellular pathways. These pathways include growth and apoptosis. Cancer can proliferate and escape from apoptosis and consequently

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metastasize [2, 3]. Cancer is one of the fatal diseases and several factors have a role in cancer incidence such as chemical, radiant, viral, and genetics [4].

Today, the present mutable compounds in foods and the environment are many agents for disease for example cancer [5]. Recently, the mortality rate of cancer is rising in many countries. Therefore, treating importance in cancer and its adversary effects has been caused to anti-mutagenesis and anticancer properties of probiotics would be considered such as acid lactic bacteria [6-9]. Probiotics are useful intestinal bacteria which have different capabilities to maintain homeostasis between the gastrointestinal tract and immune system. Also, probiotics have stimulating and potentiating effects on immunity [10, 11].

Probiotic foods have a potential advantage in a healthy body, improve the balance of micro-flora in the gastrointestinal tract, stimulate immunity, have anticancer activity, and so on [12]. Several studies showed that anticancer properties of probiotics are related to the enzymes in the human and animal colon. Thereby, probiotics intake probably affects cancer in its initial phase and stops it [13]. Studies demonstrated that cytoplasmic extraction and derived peptidoglycan from acid lactic bacteria prevent cancer cell proliferation [13]. These chemicals prevent cancer growth, as the excessive proliferation of cancerous cells is the main problem in patients with cancer. Moreover, probiotics increase cell cytotoxic activity and are utilized as a complementary drug along with chemotherapy [14].

Polysaccharides from *Lactobacillus acidophilus* kill the cancer cells by autophagy mechanism. Probiotic bacteria, especially *Lactobacillus* and *Bifidobacterium* can inhibit cancer in vivo and in vitro situations. Also, the elevated growth of *Bifidobacterium* could lead to suppressing colon carcinogenesis. Researchers believe that pH reduction of the colon by *Bifidobacterium* can block carcinogenesis substances. Moreover, probiotics intake increases natural killer (NK) cell activity and immunoglobulin levels [15, 16].

Today, *Lactobacillus* and *Bifidobacterium* are the most common microorganisms that are utilized as probiotic [17]. The *Lactobacillus* bacteria have the basil and coco-basil shape; non-spore; positive gram; negative oxidase, catalase, and indole. Besides, *Lactobacillus* strains reinforce the intestine mucosal barrier which could maintain and promote the immune status and decrease bacteria movement [18]. *Lactobacillus* and *Bifidobacterium* are the natural intestine flora and could stimulate immune responses and reduce co-

lon cancer risk. Therefore, both of them have an important role in keeping health [19]. Studies showed that metabolites produced by these bacteria have cytotoxic effects on cancer cell lines and also prevent the growth of pathogenic bacteria. In the present study, the synergistic effects of secreted molecules from *Lactobacillus* and *Bifidobacterium* on AGS and MCF-7 cell lines are evaluated.

Materials and Methods

Cell line culture

Herein, MCF-7 (NCBI No: C135) and AGS (NCBI No: C131) cell lines were provided from Pasteur Institute in Tehran, Iran. The cells were defreezing and then the cells were defrozen. The cells were counted and adjusted at the optimum concentration. After they reached 70% concentration, they were transferred into a larger flask.

Probiotic bacteria culture

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

IC50

The cytotoxicity method was utilized to determine the IC50 value of *Bifidobacterium bifidum* and *Lactobacillus acidophilus* extraction on MCF7 and AGS cell lines. Initially, 100 µL suspension was cultured in RPMI-1640 medium containing 10% FBS serum. Next, the cells were incubated at 37° C and 5% CO₂ for 24 h. Diluted extracts with different concentrations of each extraction were added to each well in triplicate. After incubation for 24, 48, and 72 h, the MTT solution (20 µL) was added to each well and incubated for 4 h. Then, the supernatant was removed and 100 µL of DMSO was dispensed to the wells. Eventually, the plates were read by ELISA reader at 590 nm wavelengths.

mRNA levels assessment

In the present study, the expression of β -actin, *Bcl2*, and *Bax* genes was evaluated in cancerous cells, and also

TNF- α gene expression in normal cells was measured by real-time PCR. First, cDNA was synthesized and RNA was extracted based on the protocol extraction of RNX plus kit. Determination of RNA quantity and concentration of MCF7 and AGS cell lines which were treated with optimum concentration of treating agent by Nano-Drop machine according to Fermentas RevertAid™ First Strand cDNA Synthesis Kit. Then, gene expression was assessed. β -Actin was used as a housekeeping gene. *Bcl2* and *Bax* gene expression levels were evaluated in the AGS cell line treated with probiotic bacteria. In addition to primers of *Bcl2* and *Bax* genes, one more pair of primer were designed for the β -actin gene. Also, the master mix (Bioneer), primers of β -actin, *Bcl2*, and *Bax* genes were used in duplicate, and *TNF- α* gene expression was assessed in normal cells.

Statistical analysis

Statistical analyses were performed by SPSS v. 16 and the results were evaluated by 1-way ANOVA test. Besides, target genes expression between control and treated samples were measured by Tukey's HSD post-hoc test. Data analysis of real-time PCR results was performed by threshold cycle and its formula is $2^{-\Delta\Delta C_t}$.

Results

IC50 value

AGS

Results of the cytotoxicity assay were evaluated. After 24 h treatment, our results showed that the treatment with

Lactobacillus acidophilus in dilutions of 1:2 and 1:4 led to a significant increase in the inhibition of cell growth in the treated cells compared with the untreated cell line as a negative control group ($P < 0.0173$). Regarding the suppression of cell growth, the results demonstrated no significant increase in treated cells compared with the negative control group in dilutions of 1:8 up to 1:32 ($P < 0.0716$). Besides, the AGS cell line treated with *Bifidobacterium* showed a remarkable increase in the suppression of cell growth as compared with the negative control group in dilutions of 1:2 up to 1:8 ($P < 0.0001$). In dilutions of 1:16 and 1:32, we did not observe any considerable increment in treated cells compared with the negative control group.

After 24 h incubation, the results of treated cells with a mixture of *Bifidobacterium bifidum* and *Lactobacillus acidophilus* extracts showed a remarkable increase in the inhibition of cell growth compared to the negative control group in the dilution of 1:2 ($P < 0.0001$) (Figure 1).

Results of treating AGS cell line with *Lactobacillus acidophilus* extract increased remarkably the inhibition of cell growth compared with the negative control group in dilutions of 1:2 up to 1:8 after 48 h treatment ($P < 0.0069$). The treatment with *Bifidobacterium bifidum* could remarkably elevate the inhibition of cell growth in dilutions of 1:2 and 1:4 compared with the negative control group ($P < 0.0013$). There was no significant difference in the treated cell line with *Bifidobacterium bifidum* extract versus the negative control group in dilutions of 1:8 up to 1:32. The obtained results from the treated cancerous cells with a mixture of two probiotic bacteria showed a significant elevation versus the negative control group in

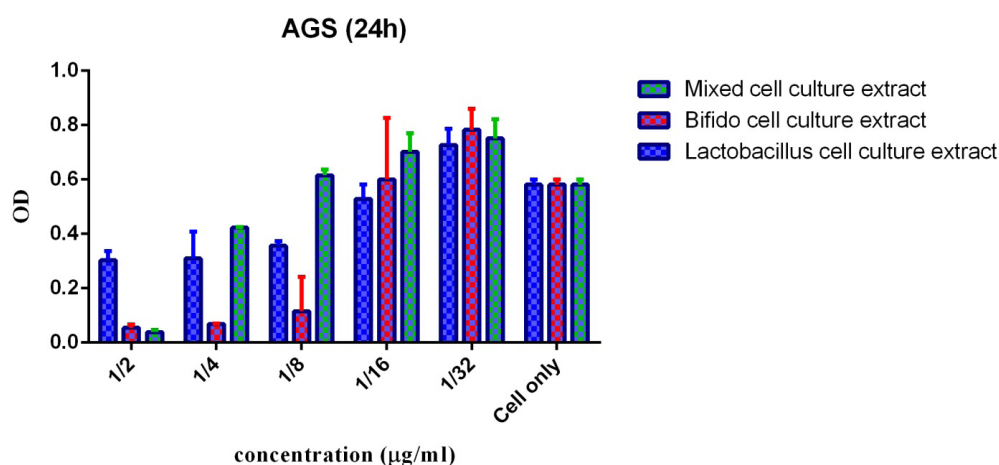


Figure 1. Cytotoxicity effect of *Lactobacillus acidophilus*, *Bifidobacterium bifidum*, or their mixture evaluated on the AGS cell line in different dilutions by MTT method after 24 hours

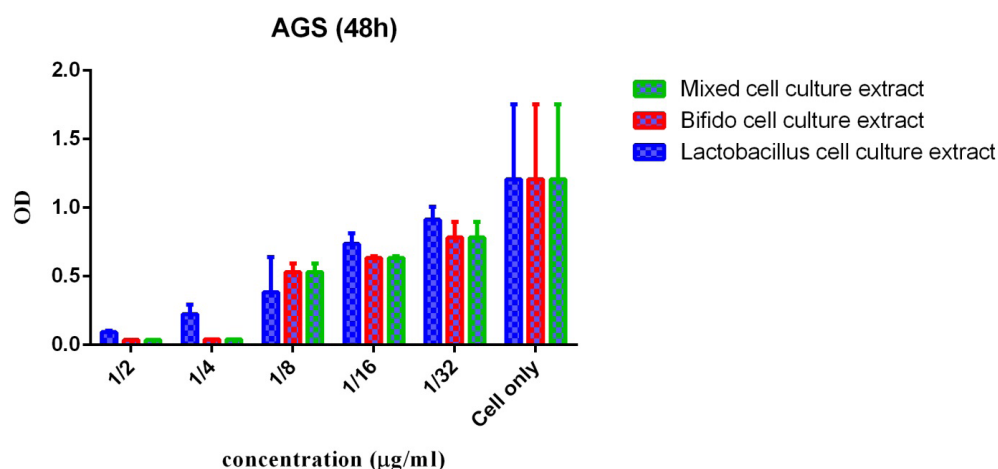


Figure 2. Cytotoxicity effect of *Lactobacillus acidophilus*, *Bifidobacterium bifidum*, or their mixture evaluated on the AGS cell line in different dilutions by MTT method after 48 hours

dilutions of 1:2 and 1:4 after 48 hours incubation time ($P < 0.0013$) (Figure 2).

The results of treated AGS cell line with *Lactobacillus acidophilus* demonstrated a remarkable suppression of cell growth versus the control group in dilutions of 1:2 and 1:4 ($P < 0.0071$). Cell growth was suppressed by the treatment with *Bifidobacterium* compared with the untreated cells in dilution of 1:2 ($P < 0.0040$). But, after 72 h treatment time, we observed no remarkable difference in the treated cells as compared with the untreated cells in dilutions of 1:4 up to 1:32 (Figure 3).

MCF-7

In dilutions of 1:2 up to 1:8, the breast cancer cells treated with *Lactobacillus acidophilus* showed a remarkable

suppression of cell growth versus the control group after 24 hours of incubation time ($P < 0.0001$). Nevertheless, in dilutions of 1:16 and 1:32, no significant difference was observed between the treated cells with *Lactobacillus acidophilus* and the negative control group. Also, there was an increase in the inhibition of cell growth when the cells were treated with *Bifidobacterium bifidum* in dilutions of 1:2 up to 1:16 compared with the control group ($P < 0.0001$). However, there was no significant difference in the treated cells with *Bifidobacterium bifidum* extract versus the untreated cell line as a control group in dilutions of 1:16 and 1:32. There was a remarkable increase in the inhibition of cell growth through the treatment of MCF-7 cell line with the mixture extracts of *Lactobacillus acidophilus* and *Bifidobacterium bifidum* compared

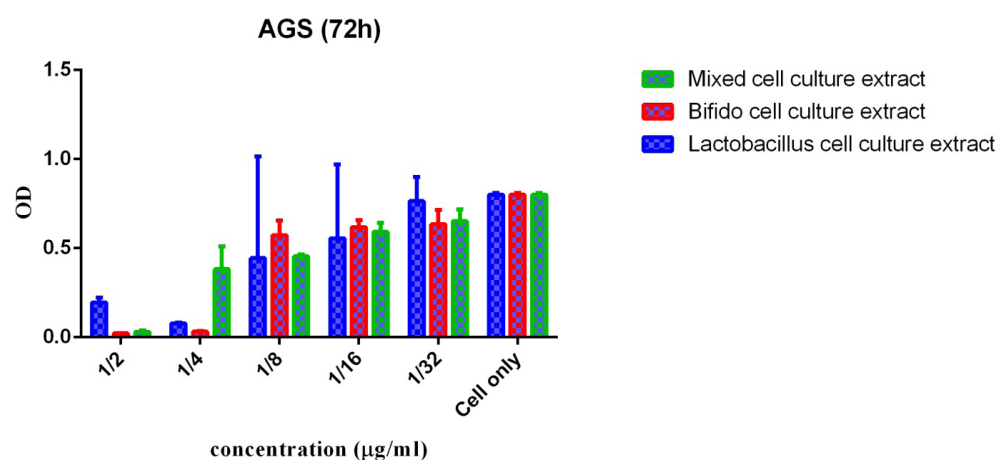


Figure 3. Cytotoxicity effect of *Lactobacillus acidophilus*, *Bifidobacterium bifidum*, or their mixture evaluated on the AGS cell line in different dilutions by MTT method after 72 hours

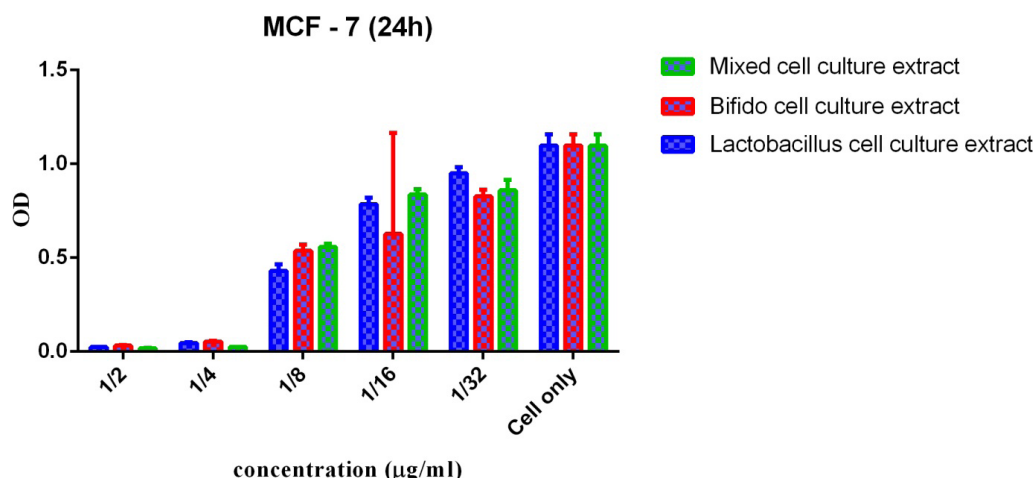


Figure 4. Cytotoxicity effect of *Lactobacillus acidophilus*, *Bifidobacterium bifidum*, or their mixture evaluated on the MCF7 cell line in different dilutions by MTT method after 24 hours

with the untreated cell line as a negative control group in dilutions of 1:2 up to 1:8 ($P < 0.0001$) (Figure 4).

Results of cytotoxicity assay after 48 h incubation time demonstrated that the treating cells with *Lactobacillus acidophilus* extract remarkably inhibited the cell growth versus the untreated cell line as a negative control group in dilutions of 1:2 up to 1:32 ($P < 0.0001$).

The treatment MCF-7 cell line with *Bifidobacterium bifidum* extract led to an increase in the inhibition of cell growth as compared with the cell line as a negative control group in dilutions of 1:2 up to 1:32 ($P < 0.0001$).

Inhibition of cell growth significantly increased after treatment by mixture extracts of *Lactobacillus acidophilus* and *Bifidobacterium bifidum* in dilutions of 1:2 up to 1:16 compared with the untreated cell line as a negative control group ($P < 0.0001$) (Figure 5).

After 72 h incubation time, the results of treatment with *Lactobacillus acidophilus* extract in dilutions of 1:2 up to 1:32 showed a significant increase compared with the untreated cell line as a negative control group ($P < 0.0001$). Also in dilutions of 1:2 up to 1:32, the results showed a significant increase in the treated cell with *Bifidobacterium bifidum* extract as compared with the untreated cell line as a negative control group

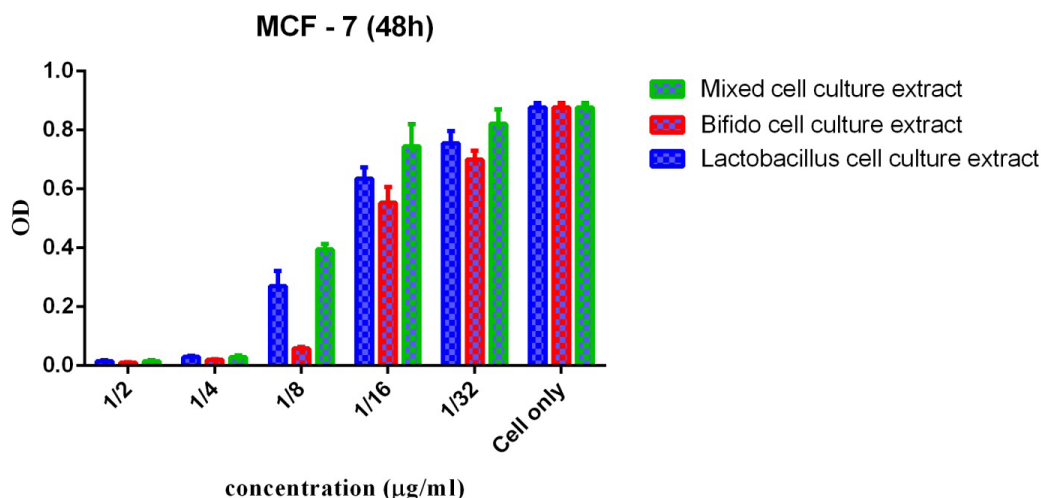


Figure 5. Cytotoxicity effect of *Lactobacillus acidophilus* and *Bifidobacterium bifidum* evaluated on the MCF7 cell line in different dilutions by MTT method after 48 hours

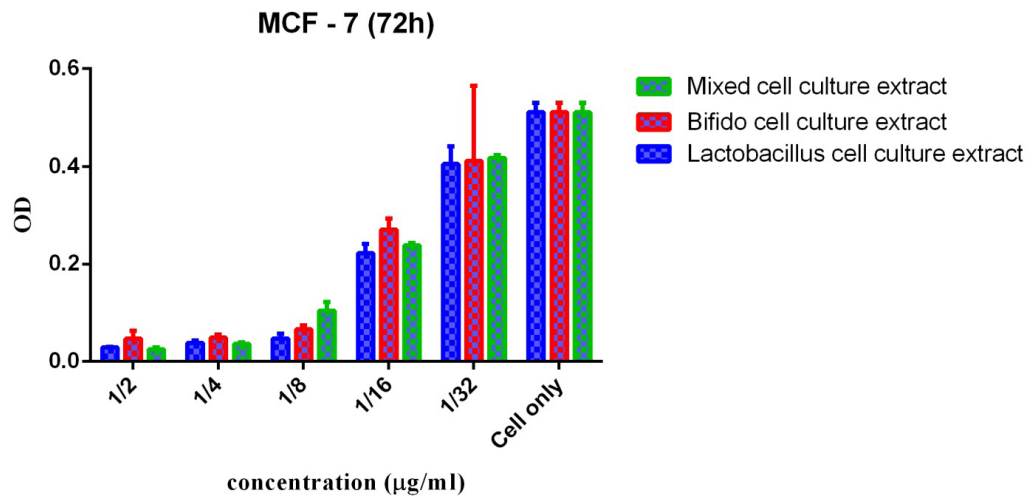


Figure 6. Cytotoxicity effect of *Lactobacillus acidophilus*, *Bifidobacterium bifidum*, or their mixture evaluated on MCF7 cell line in different dilutions by MTT method after 72 hours

($P < 0.0001$). Inhibition of cell growth increased by the treatment with the mixture extracts of *Lactobacillus acidophilus* and *Bifidobacterium bifidum* in dilutions of 1:2 up to 1:16 compared with the untreated cell line as a negative control group ($P < 0.0001$) (Figure 6).

IC50 value on PBMCs

The obtained results showed that the treated normal cells with *Lactobacillus acidophilus* in dilutions of 1:2 up to 1:32 increased significantly compared to the untreated cell line after 24 h treatment time ($P < 0.0001$). Also, in dilutions of 1:2 up to 1:32, the normal cells treated with *Bifidobacterium bifidum* showed remarkable increment

compared with the negative control which leads to an increase in the suppression of cell growth compared with the negative control group ($P < 0.0001$) (Figure 7).

The evaluation of the results showed that the treated normal cell with *Lactobacillus acidophilus* extract in dilutions of 1:2 up to 1:32 did not show any significant differences as compared with the untreated cell line as a negative control group after 48 h incubation time. Besides, the normal cells treated with *Bifidobacterium bifidum* did not show any remarkable differences compared with the negative control group in dilutions of 1:2 up to 1:32.

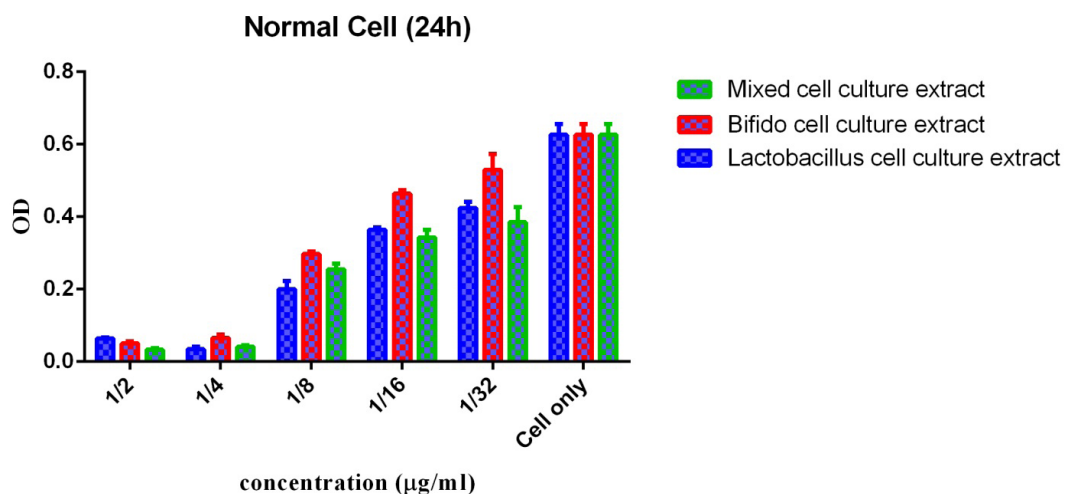


Figure 7. Cytotoxicity effect of *Lactobacillus acidophilus*, *Bifidobacterium bifidum*, or their mixture on PBMCs evaluated by the MTT method in different dilutions after 24 hours

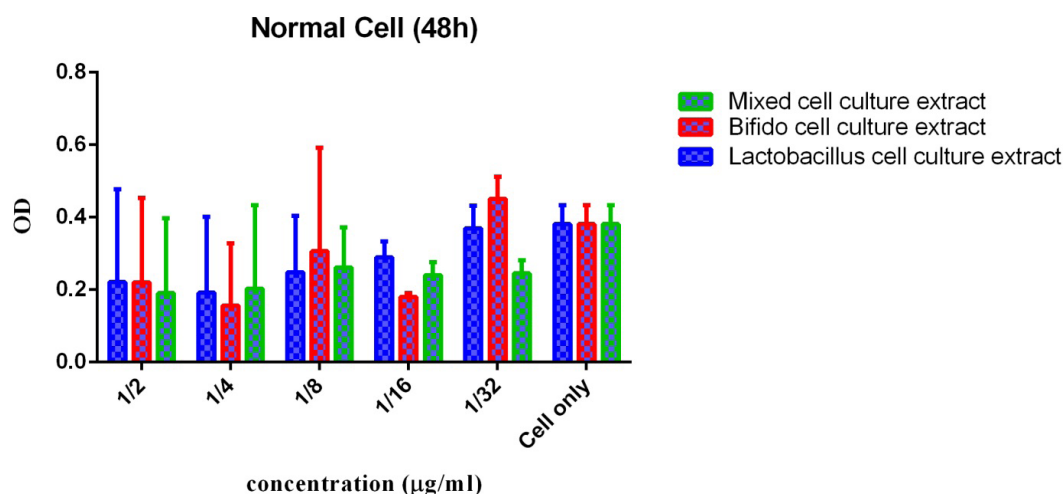


Figure 8. Cytotoxicity effect of *Lactobacillus acidophilus*, *Bifidobacterium bifidum*, or their mixture on PBMCs evaluated by the MTT method in different dilutions after 48 hours

Normal cells treated with the mixture extracts of *Lactobacillus acidophilus* and *Bifidobacterium bifidum* did not show any remarkable differences in the inhibition cell growth as compared with the untreated cell line as a negative control group (Figure 8).

The results of treatment with *Lactobacillus acidophilus* extract in dilutions of 1:2 up to 1:32 demonstrated a remarkable increase versus the control group ($P < 0.0001$). Besides, the results demonstrated a significant increase in the treated cells with *Bifidobacterium bifidum* extract in dilutions of 1:2 up to 1:16 compared with the control group ($P < 0.0001$). In dilutions of 1:2 up to 1:16, the cell growth

was suppressed by the treatment with a mixture of probiotic bacteria versus the control group ($P < 0.0001$) (Figure 9).

mRNA levels of apoptotic molecules

After 24 h treatment, the results of gene expression assessment on the AGS cell line when treated with *Lactobacillus acidophilus* showed that *Bax* gene expression increased significantly ($P < 0.0003$). The mRNA level of *Bcl2* increased but it was insignificant ($P > 0.513$). Also, after *Bcl2*, *Bax*, and β -actin gene expression assessment on the AGS cell line when treated with *Bifidobacterium bifidum* demonstrated a significant increase in *Bax* gene expression ($P < 0.0001$). The *Bax* gene expression

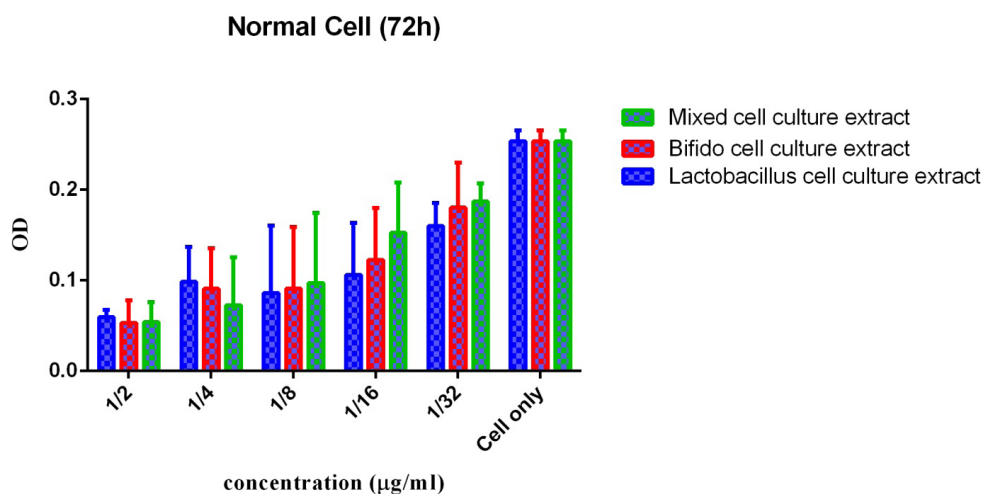


Figure 9. Cytotoxicity effect of *Lactobacillus acidophilus*, *Bifidobacterium bifidum*, or their mixture on PBMCs evaluated by the MTT method in different dilutions after 72 hours

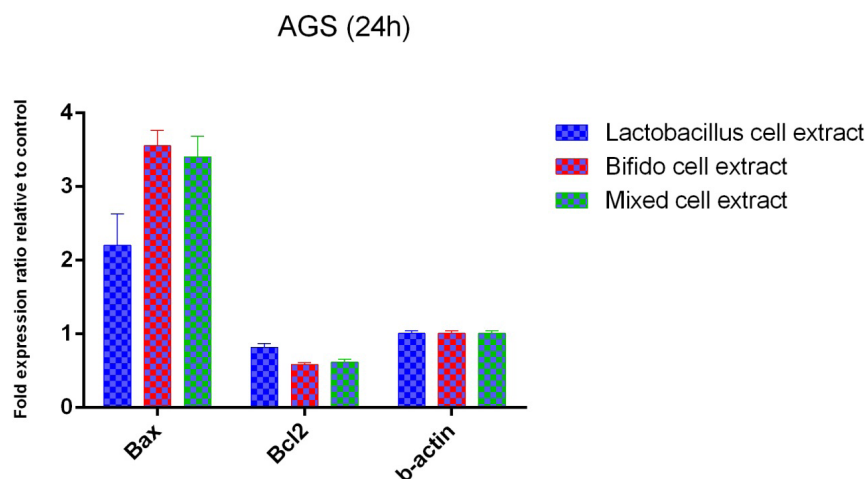


Figure 10. *Bax*, *Bcl2*, and β -actin gene expression after 24 hours treatment with *Lactobacillus acidophilus* and *Bifidobacterium bifidum* and their mixture on the AGS measured by real-time PCR

showed a remarkable increase (3.4 folds) after treatment with a mixture of *Lactobacillus acidophilus* and *Bifidobacterium bifidum* on the AGS cell line after 24 h ($P < 0.0001$) (Figure 10).

After 48 hours of treatment with *Lactobacillus acidophilus*, *Bifidobacterium bifidum*, or their mixture, the results of gene expression assessment on the AGS cell line showed significant 2.4-, 3.95-, and 3.5-fold increase in *Bax* gene expression, respectively ($P < 0.0001$) (Figure 11).

After 72 hours of treatment with *Lactobacillus acidophilus*, *Bifidobacterium bifidum*, or their mixture, the results of gene expression assessment on the AGS cell line showed significant 2.65-, 4.4- and 4.1-fold increase in *Bax* gene expression, respectively ($P < 0.0001$) (Figure 12).

After 24 hours treatment with *Lactobacillus acidophilus*, *Bifidobacterium bifidum*, or their mixture, the results of gene expression assessment on MCF-7 cell line showed significant 2.65-, 3.25- and 3.1-fold increase in *Bax* gene expression, respectively ($P < 0.0001$) (Figure 13).

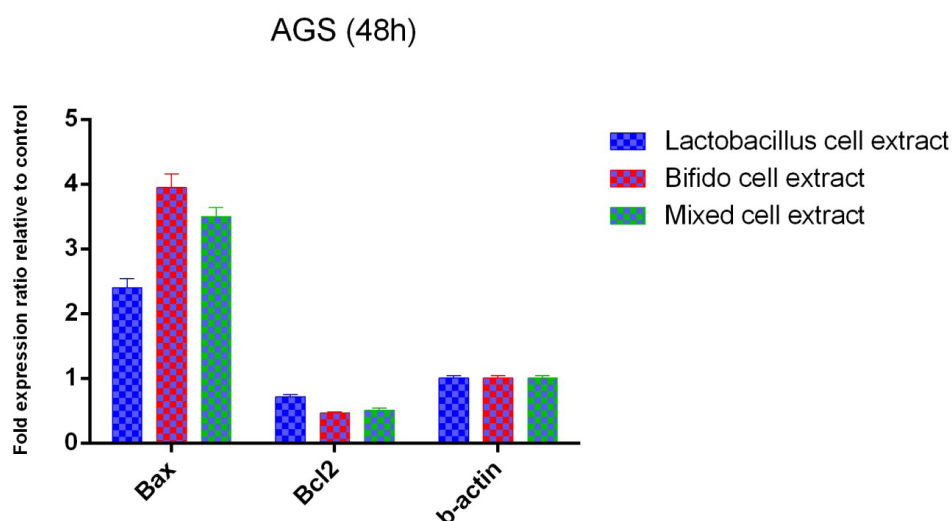


Figure 11. *Bax*, *Bcl2*, and β -actin gene expression levels after 24 h treatment with *Lactobacillus acidophilus*, *Bifidobacterium bifidum* or their mixture on the AGS measured by real-time PCR

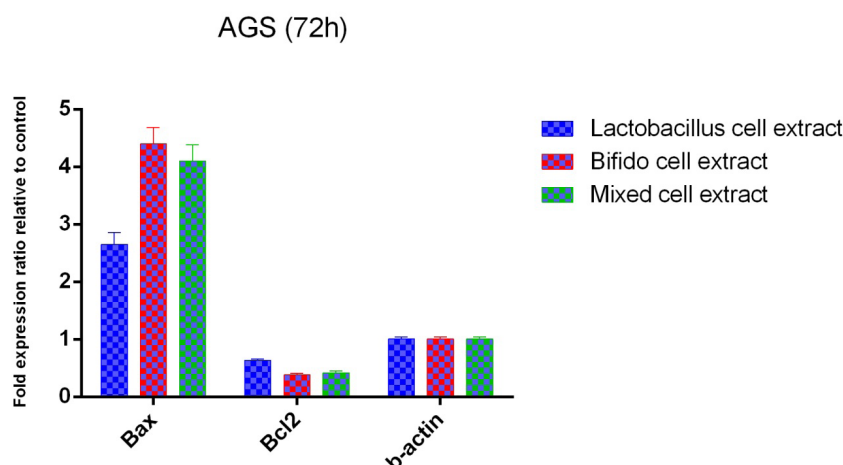


Figure 12. *Bax*, *Bcl2*, and β -actin gene expression after 72 hours treatment with *Lactobacillus acidophilus*, *Bifidobacterium bifidum*, or their mixture on the AGS cell line measured by real-time PCR

After 48 hours of treatment with *Lactobacillus acidophilus*, *Bifidobacterium bifidum*, or their mixture, the results of gene expression assessment on MCF-7 cell line showed significant 2.9-, 3.55- and 3.15-fold increase in *Bax* gene expression, respectively ($P < 0.0001$) (Figure 14).

Figure 14. *Bax*, *Bcl2*, and β -actin gene expression after 48 hours treatment with *Lactobacillus acidophilus*, *Bifidobacterium bifidum*, or their mixture on MCF-7 measured by real-time PCR

After 72 hours of treatment with *Lactobacillus acidophilus*, *Bifidobacterium bifidum*, or their mixture, the results of gene expression assessment on MCF-7 cell line showed

significant 3.3-, 3.95- and 3.5-fold increase in *Bax* gene expression, respectively ($P < 0.0001$) (Figure 15).

mRNA levels of cytokines

The obtained results of treatment with a mixture of probiotic bacteria showed a remarkable increase in $\text{IFN-}\gamma$ and *TNF- α* gene expression after 72 hours of treatment. In particular, after treatment with *Lactobacillus acidophilus*, there were 1.75- and 1.4-fold increase in $\text{IFN-}\gamma$ and *TNF- α* gene expression, respectively. Also, after treatment with *Bifidobacterium bifidum* and, there were 2.35- and 1.55-fold increase in $\text{IFN-}\gamma$ and *TNF- α* gene expression, respectively. Finally, after treatment with their mixture, there were 2.5- and 1.85-fold increase in $\text{IFN-}\gamma$ and *TNF- α* gene expression, respectively ($P < 0.0181$) (Figure 16).

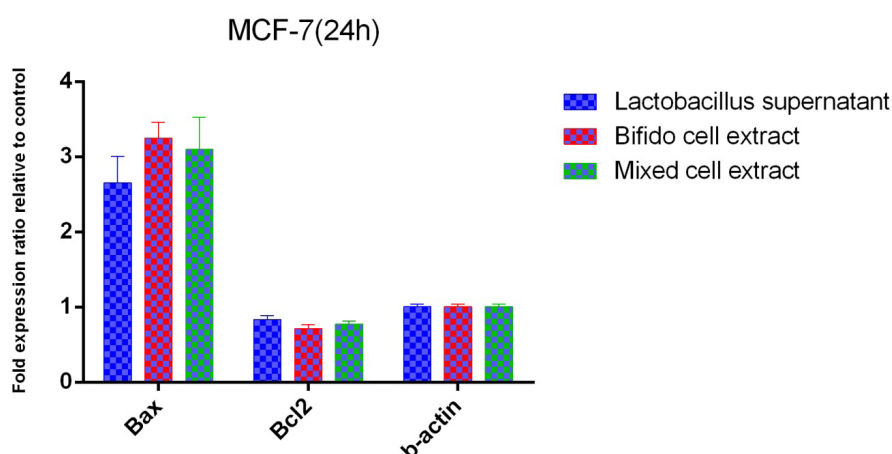


Figure 13. *Bax*, *Bcl2*, and β -actin gene expression after 24 hours treatment with *Lactobacillus acidophilus*, *Bifidobacterium bifidum*, or their mixture on MCF-7 measured by real-time PCR

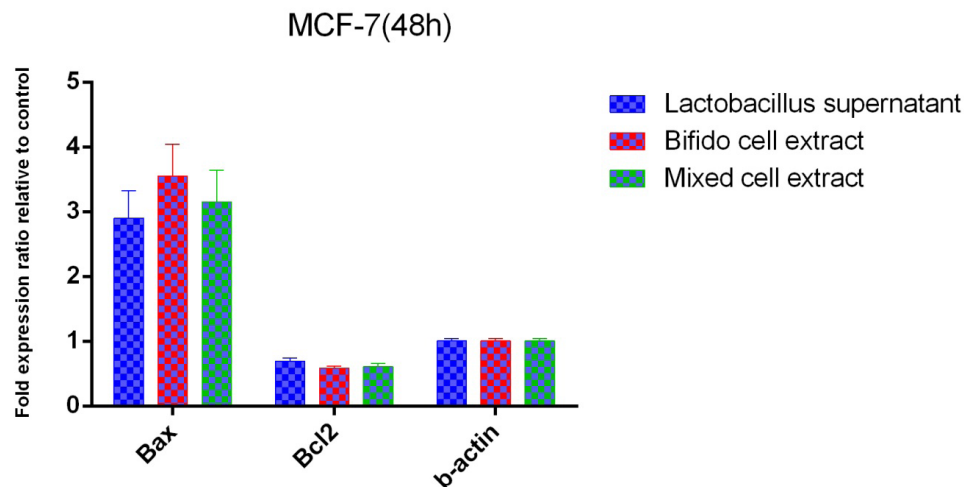


Figure 14. *Bax*, *Bcl2*, and β -actin gene expression levels after 24 h treatment with *Lactobacillus acidophilus*, *Bifidobacterium bifidum* or their mixture on the AGS measured by real-time PCR

Discussion

Various research studies have reported that apoptosis induction is a cellular pathway for removing tumor cells [20, 21]. Another mechanism is the immunity reinforcement by which tumor cells are killed through specific-tumor B and T cells [22]. In the present study, we evaluated the Th1 cytokine profile i.e., IFN- γ and TNF- α as important cytokines produced from T helper (Th)1 lymphocytes. These cytokines are a suitable index of cellular immune performance that presents an effective appraisal of cellular immunity in counteracting tumor cells [23, 24]. Additionally, various studies have

demonstrated that IFN- γ and TNF- α cytokines play a key role in the induction of the apoptosis pathway [25, 26].

Therefore, the evaluation of these two cytokines can help us to understand better the strengthening of the immune system and the induction of apoptosis. On the other hand, probiotics intake in tumor models can shift the immunity toward Th1 responses because an increase in IFN- γ and IL-12 cytokines is observed. Also in another study, the increase in inflammatory cytokines such as IL-17 and TNF- α has been observed. All these events demonstrate that probiotics can affect the immune system and potentiate cellular immunity [27]. Besides,

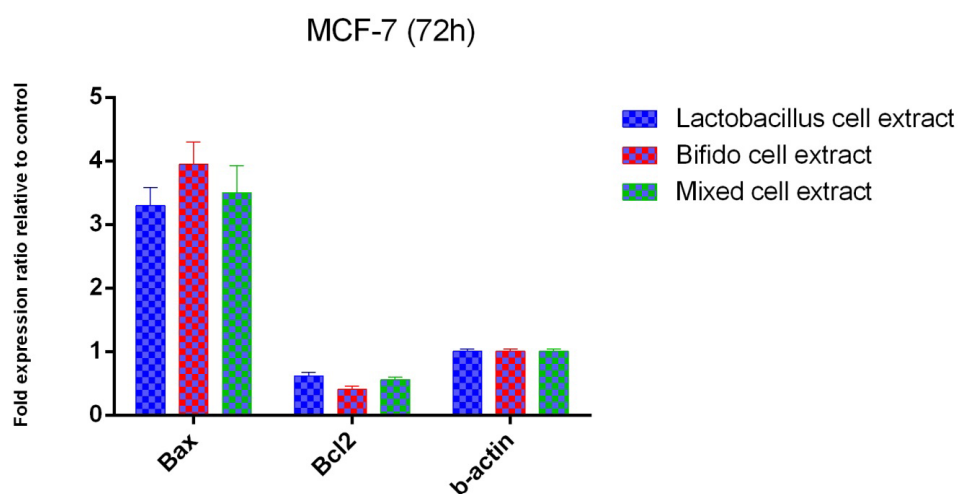


Figure 15. *Bax*, *Bcl2*, and β -actin gene expression after 72 hours treatment with *Lactobacillus acidophilus*, *Bifidobacterium bifidum*, or their mixture on MCF-7 measured by real-time PCR

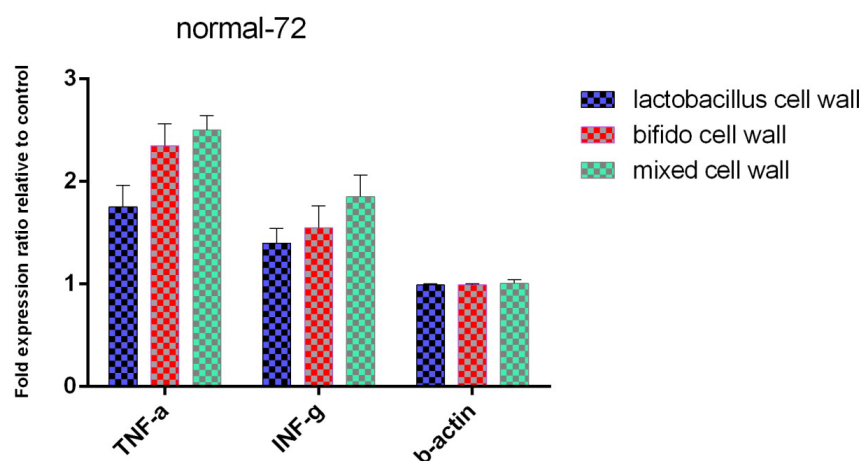


Figure 16. IFN- γ , TNF- α , and β -actin gene expression were evaluated after 72 hours treatment with *Lactobacillus acidophilus*, *Bifidobacterium bifidum*, or their mixture on PBMCs by real-time PCR

studies show that different probiotics intake such as *Lactobacillus acidophilus* and *Bifidobacterium bifidum* could stimulate Th1 responses and enhance IFN- γ , IL-12, and IL-17 cytokines and consequently suppressing tumor through the effect on CD8⁺ T cell population [28].

In the present study, our results of cytotoxicity assay showed that secreted peptides from *Lactobacillus acidophilus*, *Bifidobacterium bifidum*, or their mixture have cytotoxicity effect on MCF-7 cell line in dilutions of 1:2 up to 1:16. It seems that supernatant of these bacteria could not show a synergistic effect in cytotoxicity. Some studies also indicated that secreted supplies of probiotics display the cytotoxicity effect. Biffi et al. in another study showed that five different species of bacteria have inhibitory effects on the MCF-7 cell line and the highest effect belonged to *Lactobacillus acidophilus* and *Bifidobacterium bifidum* [29].

It seems that probiotics in liquid medium culture can induce apoptosis in many cell lines with the secretion of small peptides. Our results of the antitumor effect of *Lactobacillus acidophilus*, *Bifidobacterium bifidum*, and their mixture on the AGS cell line demonstrated a cytotoxic effect in dilutions of 1:2 up to 1:4. Likewise, another study showed that secreted factors of probiotics can suppress many types of tumor cells in the in vitro condition. Mahmudi et al. evaluated the effect of *Bifidobacterium bifidum* extract on Caco-2 cell line. Their results showed that the inhibitory effect of supernatants has a direct relation with their concentration [30].

These findings confirm our scientific study that secreted factors from *Lactobacillus acidophilus* and *Bifido-*

bacterium bifidum inhibit the growth of cancerous cells in the in vitro condition. Notably, they do not have any synergistic effects when they were used simultaneously. Also, the cytotoxicity effect of secreted factors from these bacteria and their mixture was evaluated and has been shown in dilutions of 1:2 up to 1:32, there was a cytotoxic effect on normal cells at 24 h. After 48 h, there were not any cytotoxicity effects on these cells. However, After 72 h, the cytotoxicity effect was observed in dilutions of 1:2 up to 1:16. On the other hand, apoptosis genes expression was evaluated on the AGS and MCF-7 cell lines after 24, 48, and 72 h.

We showed that microbial products of these probiotic bacteria induced the apoptosis in MCF-7 breast cancer cell and AGS gastrointestinal cancer cells through elevating *Bax* apoptotic gene expression. Additionally, TNF- α and IFN- γ cytokines were measured in normal cells after exposure to secreted factors from these bacteria and also their mix. Our results of TNF- α and IFN- γ cytokines demonstrated that extract of these bacteria alone or together could increase IFN- γ and TNF- α significantly as compared with the untreated cell and β -actin as a control group. Thus, we showed that cellular immune responses were improved through the upregulation of IFN- γ and TNF- α . Likewise, Ghadimi et al. reported that lactic acid bacteria increase Th1 cytokine (IFN- γ) in mycobacteria infection [31].

Also, Santos et al. reported the same results in which *B. toyonensis* probiotic bacterium increased IFN- γ cytokine in the vaccine system [32]. Our results are in line with the results of other studies. Asghari et al. studied the cytotoxicity effect of *Lactobacillus casei* as a probiotic bacterium on the HT-29 cancer cell line and evaluated

Bax and *Bcl2* apoptotic gene expression. Their results showed that *Bax* and *Bcl2* gene expression increased after treatment with *Lactobacillus casei* [33].

Our findings reveal that secreted factors of these bacteria may suppress the growth of tumor cells via inducing apoptosis and potentiating the immune system. *TNF- α* cytokine can inhibit angiogenesis in tumors and also induce necrosis in tumor cells. IFN- γ cytokine with various immune activities in the induction of Th1 differentiation, reinforcement of the immune system, and CD8+T cells performance can increase cytotoxicity in these cells against tumor cells [28]. According to our in vitro results, it seems that this supply induced apoptosis in cancer cells directly and could be administered orally to mice. Furthermore, some studies showed that probiotics could induce Th1 and inflammatory cytokines and potentiate immunity and promote IFN- γ and *TNF- α* cytokines [28, 34]. Therefore, in addition to apoptosis induction, the immune system is potentiated.

Conclusion

The secreted factors from the probiotic bacteria can probably remove cancer cells by apoptosis mechanism and these secreted proteins may promote cellular immunity via shift to Th1 response and inflammatory cytokines. This event implies that the apoptosis mechanism is stimulated by the products of probiotics. Due to financial constraints, we could not perform further molecular and cellular experiments such as evaluation of protein concentration, CD marker, invasive, and migration assay.

Ethical Considerations

Compliance with ethical guidelines

All ethical principles were considered in this article.

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Authors' contributions

Design, conceptualization: Mehdi Mahdavi; Performing the study: Fereshteh Kamkar; Statistical analysis: Setareh Haghighat; Write, edit and revision: All authors.

Conflicts of interest

The authors declared no conflict of interest.

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