Physiology and Pharmacology 27 (2023) 426-434
Original Article

Investigating the effects of Lactobacillus acidophilus and Lactobacillus paracasei supernatant on cervical cancer cells

Iman Samiei Mosleh[1#], Fatemeh Karami[2*#], Iman Salahshourifar[1], Maryam Tajabadi Ebrahimi[3], Mohsen Marvibaigi[4]

1. Department of Biology, Science and Research Branch, Islamic Azad University, Tehran, Iran
2. Department of Medical Genetics, Applied Biophotonics Research Center, Science and Research Branch, Islamic Azad University, Tehran, Iran
3. Department of Biology Central Tehran Branch Islamic Azad University Tehran Iran.
4. Department Biology, Kavian Institute of higher education, Mashhad, Iran

ABSTRACT

Introduction: Lactic acid bacteria, recognized as probiotics, have garnered significant attention as potential adjuvants in chemotherapy for various cancer types, including cervical cancer. In this study, we investigated the anti-cancer properties of two indigenous Iranian strains, *Lactobacillus acidophilus* and *Lactobacillus paracasei*, individually and in combination, targeting human cervical cancer cell lines compared to normal control cells.

Methods: The cytotoxic effect of *Lactobacillus acidophilus* and *Lactobacillus paracasei* supernatants, as well as their 1:1 mixture, on CaSki and HNCF PI 52 cell lines, was evaluated using the MTT assay. The apoptotic and anti-metastatic effects of these supernatants were assessed by analyzing the gene expression of *BAX/BCL2* ratio, *Caspase-3*, and *MMP2/MMP9* using Real-Time Reverse Transcriptase Polymerase Chain Reaction (RT-PCR).

Results: Significant cytotoxicity was observed in Ca Ski cells attributed to the low pH of the supernatants. The increase in the *BAX/BCL2* ratio, leading to an up-regulation of *Caspase-3*, indicated the induction of apoptosis ($P<0.001$). In addition, the expression of MMP9 significantly deceased in Ca Ski cells treated with *Lactobacillus acidophilus* ($P<0.001$) and *Lactobacillus paracasei* ($P<0.05$), while no significant difference in *MMP2* expression was observed in all samples compared to the control groups.

Conclusion: while further validation is needed, the heightened expression of apoptotic genes suggests a potential induction of apoptosis in cancer cells in response to Lactobacillus toxicity. The significant down-regulation of the *MMP9* gene emphasizes the need for comparative analyses across different cervical cancer cell lines to establish the anti-metastatic potential of these local probiotic supernatants.

Keywords:
Cervical cancer
Lactobacillus acidophilus
Lactobacillus paracasei
Probiotics

* Corresponding author: Fatemeh Karami, fatemeh.karami@srbiau.ac.ir
# These authors contributed equally in the present work.

Received 31 January 2022; Revised from 22 July 2022; Accepted 20 February 2023
Citation: Samiei Mosleh I, Karami F, Salahshourifar I, Tajabadi Ebrahimi M, Marvibaigi M. Investigating the effects of Lactobacillus acidophilus and Lactobacillus paracasei supernatant on cervical cancer cells. Physiology and Pharmacology 2023; 27: 426-434. http://dx.doi.org/10.61186/phypha.27.4.426

Introduction

The human body hosts a diverse array of microbial species crucial for overall well-being. according to the Human Microbiome Project, approximately 9% of sequenced bacterial species inhibit the urogenital tract (Mirzayi et al., 2021). The balance between genital microbiota and the host immune system is pivotal, contributing to the healthy hemostasis of the urogenital system. Disrupting this delicate equilibrium in the microbiota can pave the way for infections, eventually leading to cervical cancer.

Cervical cancer is the most prevalent gynecological malignancy and is the leading cause of cancer-related deaths in transitioning countries (Sung et al., 2021; Wen et al., 2022). High recurrence rates, especially in stages III to IVB, have amplified mortality and morbidity rates associated with cervical cancer (Chao et al., 2021). Common therapeutic approaches, such as radiotherapy and chemotherapy, not only induce cytotoxicity in normal human cells but also disrupt human microbiota, leading to various side effects for patients (Markowiak and Śliżewska 2017; Tsakmaklis et al., 2020). Thus, the quest for alternative cancer treatments or supplementary methods capable of mitigating therapy-induced side effects is becoming increasingly urgent.

Probiotics, live microorganisms administered in adequate amounts, offer numerous health benefits and contribute to maintaining a balanced host-microbial relationship. Studies indicate the probiotic food supplementation has demonstrated positive effects in preventing and managing gastrointestinal side effects induced by chemotherapy and radiation (Badgeley et al., 2021). Moreover, various studies have reported the anti-proliferative and anti-cancer effects of probiotics (Aminaei et al., 2018; Górska et al., 2019; Isazadeh et al., 2021).

The dominance of specific bacterial species such as Lactobacilli in the vaginal microbiota proves effective in inhibiting the growth of other pathogenic bacteria. These bacteria produce and release lactic acid, creating an environment with low pH that hinders the growth of pathogenes. Lactobacilli species, recognized as probiotic agents, are commonly utilized for this purpose (Chee et al., 2020). Previous studies have demonstrated that Lactobacilli not only control the growth and infectious processes of pathogens but also modulate systemic inflammation, cell proliferation, and apoptosis. However, there remains controversy in study examining the effects of different strains of Lactobacilli on cervical cancer cells (Kim et al., 2015; Motevaseli et al., 2016; Motevaseli et al., 2013). In addition, there's a lack of information regarding the mechanism of action of probiotics through gene expression assays (Wang et al., 2018). Therefore, this study aimed to evaluate the potential apoptotic and anti-metastatic effects of indigenous Iranian probiotics, specifically *Lactobacilus acidophilus* (*L.acidophilus)* and *Lactobacilus paracasei* (*L.paracasei),* on cervical cancer cell line. We conducted cell cytotoxicity assays and analyzed the expression profile of genes associated with apoptosis and metastasis as potential markers.

Materials and Methods

Cell Culture

The Ca Ski cervical cancer cell line and HNCF-PI-52 normal cervical cell line were purchased from the Iranian Biological Resource Center and Pasteur Institute of Iran, respectively. These cell lines were cultured in RPMI 1640 medium (Gibco), supplemented with 10% Fetal Bovine Serum (Gibco) and 1% penicillin/streptomycin (Gibco), and incubated at 37˚C in an atmosphere saturated with 5% CO_2.

Lactobacillus supernatant preparation

The strains *L.acidophilus* (IB-RCM-10785) and *L.paracasei* (IB-RCM-10784) were generously provided to this project by TAKGENE-company. Cultivation of these bacteria was carried out in De Man Rogosa Sharpe (MRS) broth (pH=6.5, Merck, Germany) at 37˚C for 24 hours under micro-aerophilic conditions. Subsequently, bacterial cultures (2×108 c.f.u./ml), having undergone overnight incubation, were centrifuged at 7000 rpm for 7 minutes. The resulting Lactobacilli supernatants (LS) were then filtered using a 0.2 µm membrane filter to eliminate residual bacteria and debris (Nouri et al., 2016).

Since Lactobacilli species generate lactic acid during growth, their culture media become acidic. consequently, the supernatant is expected to exhibit acidity. This acidic environment alone can induce cell lethality. To discriminate between the low pH cytotoxic effect of the supernatant and other components, MRL medium-MRS medium supplemented with lactic acid was considered as a control. Three kinds of MRL with three different pH levels were prepared: the first with a pH of 5.1, akin to the pH of *L.acidophilus* supernatant (LAS); the second

TABLE 1: Primer pair sequences which were used in RT-Real time PCR reaction.

Gene name	Primer Sequence	Product size (base pairs)
MMP2-F	AACCAGCTGGCCTAGTGATG	154
MMP2-R	CTTGGGGCAGCCATAGAAGG	
MMP9-F	CCTGGGCAGATTCCAAACCT	172
MMP9-R	GTACACGCGAGTGAAGGTGA	
Caspase-3-F	CGGCGCTCTGGTTTTCGTTA	120
Caspase-3-R	CAGAGTCCATTGATTCGCTTCC	
BAX-F	TCATGGGCTGGACATTGGAC	114
BAX-R	GAGACAGGGACATCAGTCGC	
BCL2-F	CTTTGAGTTCGGTGGGGTCA	162
BCL2-R	GGGCCGTACAGTTCCACAAA	
ACTB-F	TGGAACGGTGAAGGTGACAG	125
ACTB-R	AACAACGCATCTCATATTTGGAA	

with a pH of 4.8, similar to *L.paracasei's* supernatant's (LPS) pH; and the third with a pH of 4.9, corresponding to the pH of a 1:1 mixture of LAS and LPS supernatants. The current study was approved by ethical committee of Tehran Science and Research Branch, Islamic Azad University (IR.IAU.SRB.REC.1401.170).

MTT assay

MTT assay kit (Sigma, St. Louis, MO) was used to measure the inhibitory effect of LAS, LPS, and a 1:1 mixture on the proliferation of Ca Ski cells. A total of 10^4 cells were seeded in each well containing RPMI medium, 10 % FBS, and 1% penicillin/streptomycin. After 24 hours, the cells were treated with lactobacilli culture supernatants, MRS broth, and MRL acidic controls at concentrations of 5%, 10%, 20%, 30%, and 40% (v/v), each in triplicate. additionally, each plate underwent replication in two separate experiments. Plates were incubated for 24, 48, and 72 hours at 37˚C in an atmosphere of 5% (v/v) CO_2. Cell viability was measured using an ELISA reader (Anthons2020, version 1.8.3, UK), and analysis was performed using the following equation:

%Cell viability=Absorbance of sample-Absorbance of blank/ Absorbance of control-Absorbance of blank × 100

RNA Extraction and cDNA Synthesis

The Ca Ski and HNCF PI 52 cell lines were treated with IC50 concentrations of each supernatant in separate 6 well-plates for 24 hours. Subsequently, total RNA was extracted from both the treated and untreated cell lines using the Roche High Pure RNA Isolation Kit following the manufacturer's instructions. RNA quality and quantity were assessed using NanoDrop and electrophoresis on 0.8 % agarose gel. The isolated total RNA was then reverse transcribed into cDNA utilizing the Thermo Scientific Revert Aid Reverse Transcriptase kit according to the manufacturer's protocol.

Real-time RT-PCR

Specific primer sequences for six genes, including *BAX, BCL2*, *Caspase-3*, *MMP2,* and *MMP9* were designed and checked using the NCBI Primer-BLAST system (Table 1). Real-time reverse transcription PCR (Real-time RT-PCR) reactions were performed on a Bio-rad system (CFX Connect Real-Time PCR machine). Each polymerization reaction contained 5 ng of cDNA, 5 picomoles/µL of both forward and reverse primers, and 2X SYBR Premix Ex Taq II (Takara, Japan), adjusted to a final volume of 20 µL using ddH_2O. The thermal cycling conditions included an initial denaturation at 95ºC for 5 minutes followed by 40 cycles at 95ºC for 20 seconds and 60 ºC for 40 seconds. All reactions were carried out in duplicate, and negative controls were included in each reaction and for each primer set to identify any potential DNA contamination in reagents. Relative expression levels were determined via the comparative cycle threshold method, normalized to the expression of the *ACTB* gene. Relative quantification was calculated using the $2^{-\Delta\Delta Ct}$ method.

Statistical analysis

Graph Pad Prism V7.04 software was used for all statistical analyses in the present study. The half-maximal inhibitory concentration (IC50) was calculated through

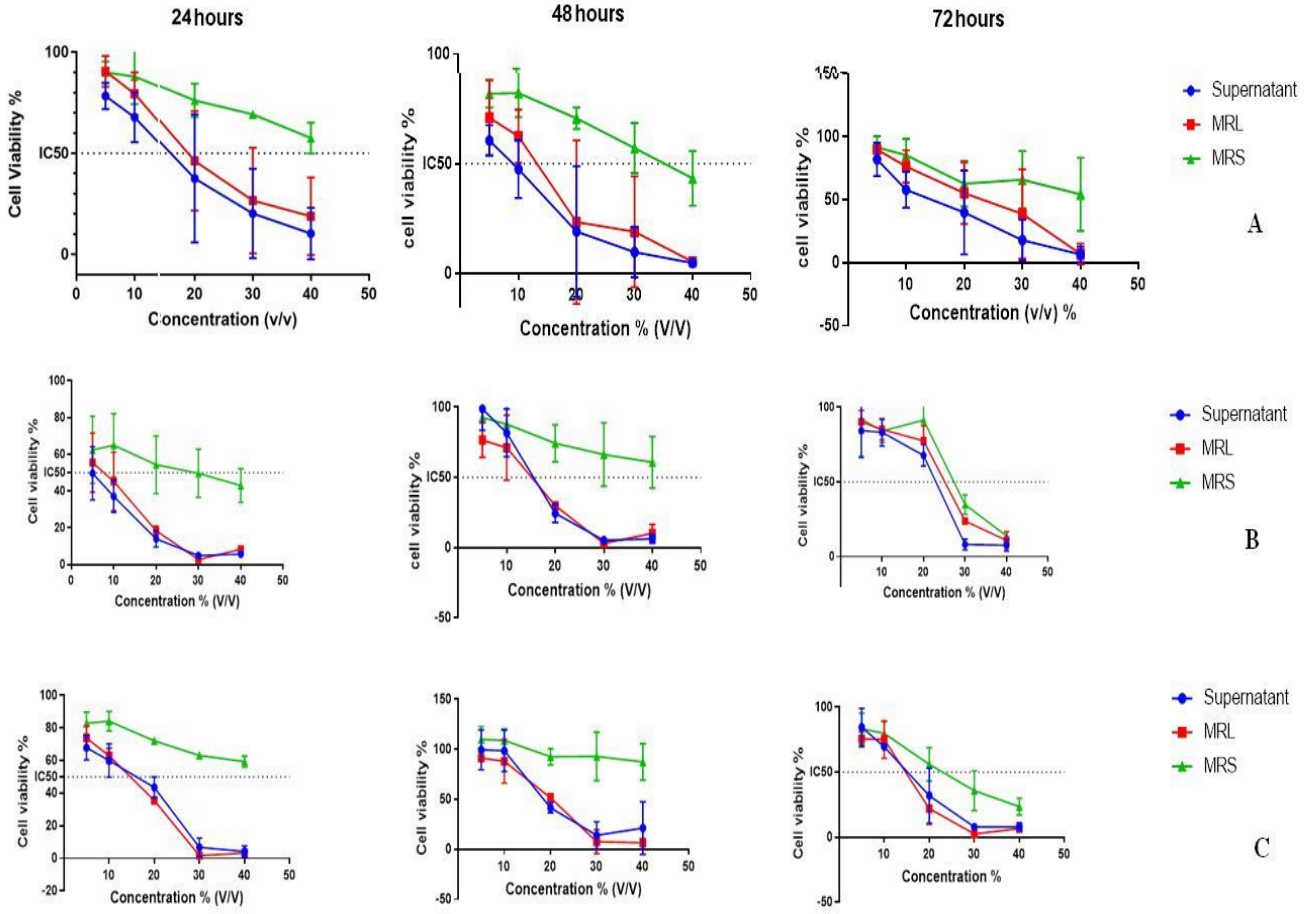

FIGURE 1. Cell viability percentage of Ca Ski cells treated with different concentrations of supernatant of A: *L.acidophilus*, B: *L.paracasei* and C: mixture of *L.paracasei* and *L.acidophilus.*

non-linear regression. Normality distribution and variance homogeneity were examined across different analysis groups using the Shapiro-Wilk test and Brown-Forsythe test, respectively.

To compare results between groups, either a standard one-way or two-way ANOVA test was implicated based on the dimensions of the data. Tukey's Honestly-Significant-Difference (TukeyHSD) Test was utilized to discern differences between specific groups. $P<0.05$ was assumed as the significance threshold.

Results

Effects of L.acidophilus and L.paracasei and a mixture of them (1:1) on Ca Ski cell line viability

After treatment of the Ca Ski cell line with the three samples of supernatant along with their respective MRL and MRS controls for 24, 48, and 72 hours, cell viability rates were estimated through the MTT test. The IC50 for each supernatant was calculated using non-linear regression. Figures 1-A, B and C illustrate cell viability post-treatment with each sample at different time points and concentrations. The corresponding IC50 values for each sample at specific time points are also shown in the figures (Figures 1-A, B, C).

Although 50% reduction in cancer cell viability was observed at low concentrations, no significant difference was noted in the lethality induced by MRL (acidic control) compared to all kinds of supernatants ($P>0.05$). Consequently, cell totoxicity appeared to be predominantly associated with the acidic environment.

Effects of L.acidophilus and L.paracasei and mix of them (1:1) on HNCF PI 52 cell line viability

After treating the HNCF PI 52 with the three supernatant types and conducting the MTT assay, IC50 values were calculated for each sample at specific time intervals using the non-linear regression model. As expected, the rate of cell viability for samples treated with the supernatants in this study was not significantly different from those treated with MRL ($P>0.05$) (Figures 2-A, B, C).

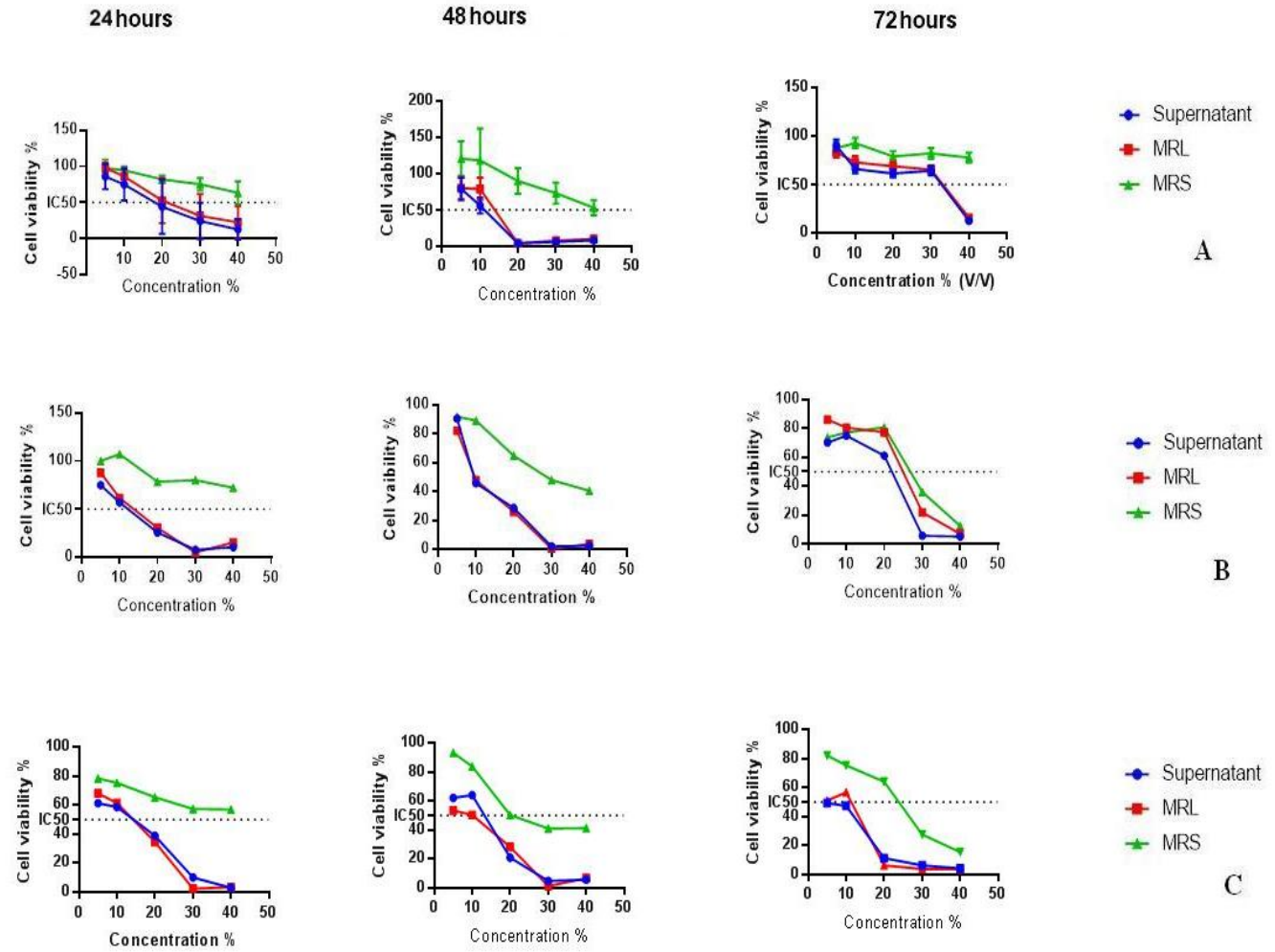

FIGURE 2. Cell viability percentage of HNCF-PI-52 cells treated with different concentrations of supernatant of A: *L.acidophilus*, B: *L.paracasei* and C: mixture of *L.paracasei* and *L.acidophilus*.

MMP2, MMP9, Caspase-3, BCL2 and BAX genes expression in Ca Ski cells

The Ca Ski cell line was treated with the 24-hour IC50 concentration of each supernatant (LAS=13.8%, LPS=6.95%, Mix=11.25%), following which alterations in candidate gene expression were assessed through RT-Real time PCR. Among the apoptotic genes analyzed, *Caspase-3* and *BAX* exhibited significant up-regulation (P<0.001) compared to the untreated control Ca Ski cell line. Notably, treatment with the mix of LAS and LPS (1:1) showed no significant difference in *BAX* expression, while the *BCL2* gene was significantly down-regulated (P<0.001). In addition, no significant difference in *MMP2* expression level was observed after treatment with all three kinds of probiotic supernatants. Conversely, significant down-regulation of *MMP9* expression was demonstrated in samples treated with LAS (P<0.001) and LPS (P<0.01). Nevertheless, *MMP9* expression remained largely unchanged following treatment with the mix of LPS and LAS (1:1) (Figure 3-A).

MMP2, MMP9, Caspase-3, BCL2, and BAX genes expression in HNCF PI 52 cells

Similarly to the Ca Ski treatment, HNCF PI 52 cells were exposed to IC50 concentrations of each supernatant (LAS= 11.25%, LPS=16.78%, Mix=10.63%). After 24 hours, RNA was extracted from the treated samples to analyze candidate gene expression levels. *BAX* exhibited significant up-regulation while *BCL2* showed significant down-regulation following treatment with all three supernatant types (P<0.001). Additionally, *Caspase-3* expression meaningfully increased after treatment with all supernatants (P<0.001). *MMP9* was down-regulated following the LAS treatment (P<0.001) while there was no change in the expression of the *MMP2* gene after treatment with any of the supernatants, and the *MMP9* gene remained unchanged following treatment with LPS and the Mix (Figure 3-B).

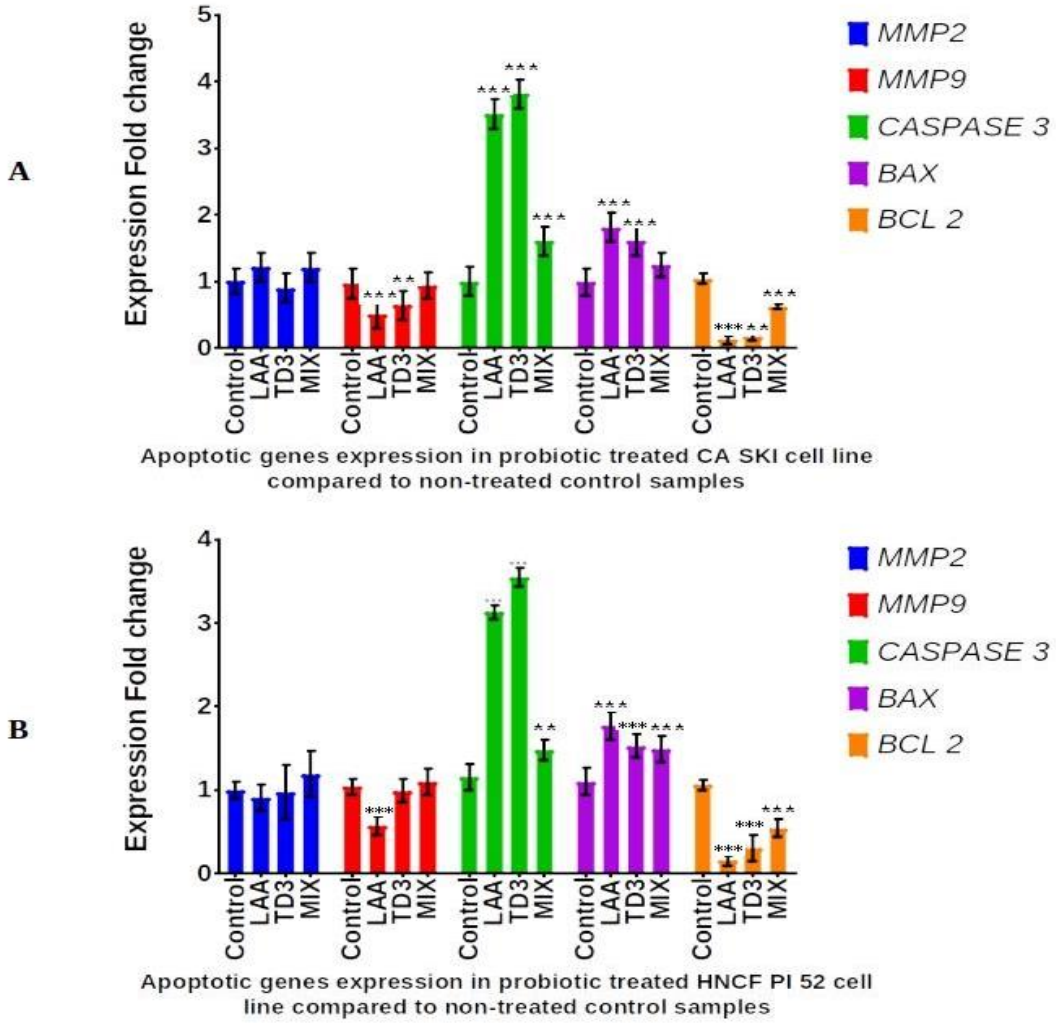

FIGURE 3. The effect of *Lactobacilli* supernatant on the mRNA expression level of *MMP2*, *MMP9*, *BCL2*, *BAX* and *Caspase-3* genes in the treated Ca Ski (A) and HNCF PI 52 (B) cells with *L.paracasei* (TD3), *L.acidophilus* (LAA) and Mix of them (MIX). *** = $P<0.001$, **= $P<0.01$ and *= $P<0.05$, MRS: De Man Rogosa Sharpe, MRL: MRS with lactic acid.

Discussion

Herein, it was demonstrated that *L.acidophilus* and *L.paracasei* supernatants, along with their mixture were significantly cytotoxic to neither CaSki cells nor HNCF-PI 52 normal cervical cells and, the observed toxicity in cancer cells was probably due to the lactic acid released in supernatants by *L.acidophilus* and *L.paracasei*. considering the possibility of low pH inducing apoptosis, the study evaluated key genes related to apoptosis initiation in response to these probiotics supernatants (Sharma et al., 2015). The increased expressions of the *BAX/BCL2* ratio and *Caspase-3* in both cancerous and normal cell lines post-treatment suggest a potential triggering of apoptosis in response to LS treatment. Moreover, no significant alteration in *MMP2* expression was observed in both cell lines following treatment. However, *MMP9* down-regulation in both cell lines treated with *L.acidophilus* and in the Ca Ski cell line treated with both Lactobacillus strains indicates an anti-metastatic effect and provides additional support for the induction of apoptosis by LS.

Contrary to our results and the study by Kim et al., which involved treating Hela and Ca Ski cell lines with *L. casei*, some other studies have described cytotoxic effects of the supernatants of other Lactobacillus species on cervical cancer cells (Kim et al., 2015). Motevaseli et al., demonstrated that the culture supernatant and extract of two common vaginal Lactobacilli, including *Lactobacillus gasseri* and *Lactobacillus crispatus*, were cytotoxic to the HeLa cell line. However, this cytotoxic effect was associated with the suppression of apoptosis, as evidenced by down-regulation of the *Caspase-3* gene, which contradicts our findings (Motevaseli et al., 2013). Riaz Rajoka et al., showcased the anticancer ef-

fect of three *Lactobacillus casei* and *paracasei* strains on HeLa cells, emphasizing their impact on increasing the expressions of *BAX*, *BAD*, *Caspase-3*, *Caspase-8*, and *Caspase-9* genes while decreasing *BCl-2* gene expression (Riaz Rajoka et al., 2018). our consistent findings in the present study regarding the increased *BAX*/*BCl-2* ratio, *MMP9,* and *Caspase-3* expression reinforce the potential pro-apoptotic effect of *Lactobacillus paracasei* supernatant on cervical cancer cells. Wang et al., demonstrated the anti-proliferative effect of three different lactobacillus strains (*L. crispatus*, *L. jensenii*, and *L. gasseri*) on the Ca Ski cell line, mediated by modulating Human Papilloma virus (HPV) and cell cycle-related oncogenes (Wang et al., 2018). Their findings partially align with another study analyzing the effects of *L. crispatus* and *L. rhamnosus* supernatants on the Hela cell line, indicating a decrease in *HPV E6* oncogene expression while observing decreased expression of *Caspase-3* and two autophagy-related genes (Kim et al., 2015). In another study, treatment with *L. crispatus* supernatant on Hela and HT-29 cell lines replicated the down-regulation of *Caspase-3*,

along with decreased expression of *MMP-2* and *MMP-9* genes, consistent with the our present findings regarding *MMP-9* following treatment with *L.acidophilus* and *L.paracasei* (Nouri et al., 2016). Although the decrease in *Caspase-3* expression alone might not be enough to discuss the effect of Lactobacillus supernatant on apoptosis initiation, it seems that different strains of Lactobacillus behave differently concerning apoptosis genes in various cervical cancer cell lines. Li et al. revealed that treating two primary cervical cancer cell lines (HeLa and U14) with *Lactobacillus debrueckii subsp. Lactis* was significantly associated with an increase in *E-cadherin* expression, an anti-metastatic gene (Li et al., 2017). Although research on the effect of lactobacillus supernatants on metastatic genes in different cervical cell lines is limited, the common finding of *MMP-9* down-regulation aligns with our results. It was found that Ca Ski cell lines exhibited the highest expression pattern of *MMP-9* compared to Hela and SiHa cervical cancer cell lines (Schröpfer et al., 2010), indicating that significant down-regulation of *MMP9* following treatment with *L.acidophilus* and *L.paracasei* supernatants could remarkably suppress cancer cell invasion and induce apoptosis. However, one notable finding of our study is the non-significant decrease in cervical cancer cell proliferation despite the overexpression of key pro-apoptotic genes following treatment with LS. This could be interpreted as apoptosis-induced cell proliferation, a compensatory mechanism identified in breast, cervical cancer, and neuroblastoma cells, enabling surviving cancer cells to proliferate (Ryoo and Bergmann 2012). This is in line with a slight increase or no change in the cell viability percentage after 72 hours of treatment of Ca Ski cells, especially with L.paracasei and the mix of both Lactobacillus. *Caspase-3* up-regulation has been shown as pivotal in the repopulation of cancer cells following radiotherapy and was suggested as a biomarker of radiotherapy response (Huang et al., 2011). In addition, analyzing apoptotic gene expression at the protein level is essential to confirm apoptosis-induced Ca Ski cell proliferation in response to *L.acidophilus* and *L.paracasei* supernatants.

The present study is one of the limited assays which have tested the effect of probiotic supernatant on normal cervical cell line, as well. Probiotic supplements are frequently used for preventing and treating vaginal and cervical infections and cervical cancer. In this regard, investigating the impact of these supplements on normal cell chemistry and physiology is as important as studying their effects on cancer cells. In the present study, although the probiotic supernatants were not toxic to normal cells, they were associated with increased expression of the *BAX*/*Bcl2* ratio and therefore apoptosis. To the best of our knowledge, this is the primary study demonstrating the apoptotic effect of Lactobacillus strains on normal cervical cells. Sadeghi-Aliabadi et al., demonstrated the anti-proliferative effect of *Lactobacillus rhamnosus* and *Lactobacillus plantarum* on two human colon cancer cell lines and normal cells (Sadeghi-Aliabadi et al., 2014). Of note, the best anti-cancer agents should protect normal cells from apoptosis while maintaining a healthy proliferation rate. This aspect should be considered in selecting Lactobacillus strains for virginal and oral supplements targeting cervical cancer (Gerl and Vaux 2005). However, inducing pro-apoptotic effects by probiotics could serve as a natural treatment for healing cervical or vaginal lesions, which might be afforded by our local probiotic strains. Further studies are necessary to determine the precise role of local Iranian probiotics on cervical cancer cells, considering protein and epigenetic analysis of apoptotic and metastatic genes.

Acknowledgment

We're about to thank Prof. Modarressi, TAKGEN Company, for presenting the Iranian local probiotic.

Conflict of interest

There is no conflict of interest among authors and the project was supported by private funding.

References

Aminaei M, Karami F, Marvibaigi M, Sotoodehnejadnematalahi F, Tajabadi Ebrahimi M. Primary evidence on the potential of Lactobacillus paracasei in treatment of hepatocellular carcinoma. Food and Health 2018; 3: 27-9.

Badgeley A, Anwar H, Modi K, Murphy P, Lakshmikuttyamma A. Effect of probiotics and gut microbiota on anti-cancer drugs: Mechanistic perspectives. Biochim Biophys Acta Rev Cancer 2021; 1875: 188494. https://doi.org/10.1016/j.bbcan.2020.188494

Chao X, Song X, Wu H, You Y, Wu M, Li L. Selection of treatment regimens for recurrent cervical cancer. Front Oncol 2021; 11: 618485. https://doi.org/10.3389/fonc.2021.618485

Chee WJY, Chew SY, Than LTL. Vaginal microbiota and the potential of Lactobacillus derivatives in maintaining vaginal health. Microb Cell Fact 2020; 19: 203. https://doi.org/10.1186/s12934-020-01464-4

Gerl R, Vaux DL. Apoptosis in the development and treatment of cancer. Carcinogenesis 2005; 26: 263-70. https://doi.org/10.1093/carcin/bgh283

Górska A, Przystupski D, Niemczura M J, Kulbacka J. Probiotic bacteria: A promising tool in cancer prevention and therapy. Curr Microbiol 2019; 76: 939-949. https://doi.org/10.1007/s00284-019-01679-8

Huang Q, Li F, Liu X, Li W, Shi W, Liu FF, et al. Caspase 3-mediated stimulation of tumor cell repopulation during cancer radiotherapy. Nat Med 2011; 17: 860-6. https://doi.org/10.1038/nm.2385

Isazadeh A, Hajazimian S, Shadman B, Safaei S, Babazadeh Bedoustani A, Chavoshi R, et al. Anti-cancer effects of probiotic lactobacillus acidophilus for colorectal cancer cell line Caco-2 through apoptosis induction. Pharm Sci 2021; 27: 262-7. https://doi.org/10.34172/PS.2020.52

Kim SN, Lee WM, Park KS, Kim JB, Han DJ, Bae J. The effect of Lactobacillus casei extract on cervical cancer cell lines. Contemp Oncol (Pozn) 2015; 19: 306-12. https://doi.org/10.5114/wo.2014.45292

Li X, Wang H, Du X, Yu W, Jiang J, Geng Y, et al. Lactobacilli inhibit cervical cancer cell migration in vitro and reduce tumor burden in vivo through upregulation of E-cadherin. Oncol Rep 2017; 38: 1561-8. https://doi.org/10.3892/or.2017.5791

Markowiak P, Śliżewska K. Effects of probiotics, prebiotics, and synbiotics on human health. Nutrients 2017; 9. https://doi.org/10.3390/nu9091021

Mirzayi C, Renson A, Zohra F, Elsafoury S, Geistlinger L, Kasselman LJ, et al. Reporting guidelines for human microbiome research: the STORMS checklist. Nat Med 2021; 27: 1885-92. https://doi.org/10.1038/s41591-021-01552-x

Motevaseli E, Azam R, Akrami SM, Mazlomy M, Saffari M, Modarressi M H, et al. The effect of lactobacillus crispatus and lactobacillus rhamnosus culture supernatants on expression of autophagy genes and HPV E6 and E7 oncogenes in the HeLa cell line. Cell J 2016; 17: 601-7.

Motevaseli E, Shirzad M, Akrami SM, Mousavi AS, Mirsalehian A, Modarressi M H. Normal and tumour cervical cells respond differently to vaginal lactobacilli, independent of pH and lactate. J Med Microbiol 2013; 62: 1065-72. https://doi.org/10.1099/jmm.0.057521-0

Nouri Z, Karami F, Neyazi N, Modarressi MH, Karimi R, Khorramizadeh MR, et al. Dual anti-metastatic and anti-proliferative activity assessment of two probiotics on HeLa and HT-29 cell lines. Cell J 2016; 18: 127-34.

Riaz Rajoka MS, Zhao H, Lu Y, Lian Z, Li N, Hussain N, et al. Anticancer potential against cervix cancer (HeLa) cell line of probiotic Lactobacillus casei and Lactobacillus paracasei strains isolated from human breast milk. Food Funct 2018; 9: 2705-15. https://doi.org/10.1039/C8FO00547H

Ryoo HD, Bergmann A. The role of apoptosis-induced proliferation for regeneration and cancer. Cold Spring Harb Perspect Biol 2012; 4: a008797. https://doi.org/10.1101/cshperspect.a008797

Sadeghi-Aliabadi H, Mohammadi F, Fazeli H, Mirlohi M. Effects of Lactobacillus plantarum A7 with probiotic potential on colon cancer and normal cells proliferation in comparison with a commercial strain. Iran J Basic Med Sci 2014; 17: 815-9.

Schröpfer A, Kammerer U, Kapp M, Dietl J, Feix S, Anacker J. Expression pattern of matrix metalloproteinases in human gynecological cancer cell lines. BMC Cancer 2010; 10: 553. https://doi.org/10.1186/1471-2407-10-553

Sharma V, Kaur R, Bhatnagar A, Kaur J. Low-pH-induced apoptosis: role of endoplasmic reticulum stress-induced calcium permeability and mitochondria-dependent signaling. Cell Stress Chaperones 2015; 20: 431-40. https://doi.

org/10.1007/s12192-014-0568-6

Sung H, Ferlay J, Siegel R L, Laversanne M, Soerjomataram I, Jemal A, et al. Global cancer statistics 2020: GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 countries. CA Cancer J Clin 2021; 71: 209-49. https://doi.org/10.3322/caac.21660

Tsakmaklis A, Vehreschild M, Farowski F, Trommer M, Kohler C, Herter J, et al. Changes in the cervical microbiota of cervical cancer patients after primary radio-chemotherapy. Int J Gynecol Cancer 2020; 30: 1326-30. https://doi.org/10.1136/ijgc-2019-000801

Wang KD, Xu DJ, Wang BY, Yan DH, Lv Z, Su JR. Inhibitory effect of vaginal lactobacillus supernatants on cervical cancer cells. Probiotics Antimicrob Proteins 2018; 10: 236-42. https://doi.org/10.1007/s12602-017-9339-x

Wen H, Guo Q-H, Zhou X-L, Wu X-H, Li J. Genomic profiling of chinese cervical cancer patients reveals prevalence of DNA damage repair gene alterations and related hypoxia feature. Front Oncol 2022; 11: 792003. https://doi.org/10.3389/fonc.2021.792003