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ORIGINAL RESEARCH ARTICLE

Extraction of Probiotic Bacteria from Camel Milk and Its Effect on Liver Cancer

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Abstract

Background Nowadays, the use of natural and alternative therapies for the prevention and treatment of cancer, including probiotics such as lactic acid bacteria, has gained increasing attention. This study aimed to investigate the anticancer, pro-apoptotic, and antioxidant effects of lactic acid bacteria isolated from camel milk in Mashhad on liver cancer, as well as their impact on the expression of BAX and BCL-2 genes using the Real-time PCR method.

Methods Camel milk samples were collected from Mashhad, and the isolated strains were identified using biochemical tests. The anticancer, anti-apoptotic, and antioxidant effects of the isolated LAB were evaluated using laboratory tests, including the MTT assay and the DPPH radical scavenging method. The effect of isolated LAB on BAX and BCL-2 gene expression in HCC cells was investigated using real-time PCR.

Results Eight Lactobacillus strains with probiotic properties were identified. Two strains, A4 and A8, had the highest probiotic activity. The DPPH radical scavenging activity for A4 was between 2–23% and for A8 between 2–19%. Strains A4 and A8 showed time- and dose-dependent toxicity on HepG2 hepatocytes. The IC_{50} values for the strain A4 were 30.66 µg/mL at 48 hours and 51.89 µg/mL at 72 hours, while for the strain A8, they were 32.03 µg/mL at 48 hours and 67.29 µg/mL at 72 hours, respectively. Caspase activity increased in hepatocytes after treatment with A4 and A8.

Conclusion The findings of this study demonstrated the anticancer, anti-apoptotic, and antioxidant effects of LAB isolated from camel milk in Mashhad on liver cancer cells and their influence on BAX and BCL-2 gene expression.

Keywords BAX, BCL-2, Camel milk, Lactic acid, Liver Neoplasms

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1 Introduction

According to studies, the progression toward cancer is often associated with impaired immune system function. Hwang et al. reported that chronic inflammation in the body correlates with the initiation of cancer cell proliferation, tumor progression, and metastasis. Cytokine secretion during these phases can either inhibit cancer cell growth or, conversely, exacerbate or suppress the response to treatment.[1]

Hepatocellular carcinoma (HCC) is a common and aggressive form of liver cancer, characterized by a high mortality rate and limited therapeutic options. Recent research has focused on lactic acid bacteria (LAB) isolated from camel milk and their potential effects on HCC cells due to the promising therapeutic properties of these microorganisms. Studies have shown that LAB exhibit notable anticancer, pro-apoptotic, and antioxidant properties, suggesting their potential application in cancer treatment.[2]

Camel milk is rich in bioactive compounds, including lactoferrin, lysozyme, and various peptides, which exert antioxidant and anti-inflammatory effects. These components have been shown to modulate oxidative stress and inflammation—both critical factors in cancer development. The antioxidant activity of camel milk is largely attributed to its high vitamin C content and specific proteins that reduce reactive oxygen species (ROS), thereby protecting cells from oxidative damage.[3]

In the context of HCC, research indicates that LAB can modulate the expression of key apoptosis-regulating genes, such as BAX and BCL-2. BAX promotes apoptosis, whereas BCL-2 inhibits it. Therefore, a favorable therapeutic outcome is associated with an increased BAX/BCL-2 ratio. Evidence suggests that LAB isolated from camel milk can upregulate BAX expression while downregulating BCL-2 in HCC cells. This shift not only promotes apoptosis in cancer cells but also contributes to the suppression of tumor growth.[4]

Furthermore, antibodies in camel milk (IgG) have demonstrated significant anti-tumor effects in diethylnitrosamine (DENA)-induced liver cancer. Mice with HCC were administered camel milk IgG daily for four weeks, which significantly reduced liver damage markers and oxidative stress while improving antioxidant status. Morphological changes in hepatocytes were attenuated, and the expression of glutathione S-transferase placental form (GST-P) was decreased. These findings suggest that purified immunoglobulins from camel milk may help improve liver function and reduce oxidative stress in HCC-bearing mice.[5]

The aim of this study is to investigate the anticancer, pro-apoptotic, and antioxidant effects of LAB isolated from camel milk in Mashhad using real-time PCR and ELISA on liver cancer cell lines.

2 Methods

Camel Milk Sample Collection

In this study, camel milk samples were collected from Khorasan Razavi province (Ehsan House Complex, “A Window to Pure Life”) while maintaining the cold chain and then transferred to the laboratory. For bacterial culture, selective de Man, Rogosa, and Sharpe (MRS) broth and agar media were used. After collection, the camel milk samples were homogenized using a vortex mixer to isolate bacteria present in the milk. Bacterial suspensions were prepared from the milk samples using sterile PBS buffer. Ten mL of the milk sample was transferred to 100 mL of sterile PBS and gently shaken to detach microorganisms. After 30 minutes, 10 mL of the resulting suspension was inoculated into MRS broth medium. The inoculated samples were incubated at 37°C for 24 hours.[2] Isolated bacteria were then purified and subjected to biochemical tests for preliminary identification.[6]

The ability of selected isolates to produce antimicrobial compounds against standard pathogenic bacteria was assessed by measuring the diameter of the inhibition zone. For this, 200 µL of an active culture of Acinetobacter baumannii ATCC 14606 and Klebsiella pneumoniae ATCC 13883 were transferred to Falcon tubes containing Mueller-Hinton broth and incubated at 37°C until reaching a turbidity equivalent to 0.5 McFarland standard. The appearance of clear inhibition zones was recorded as evidence of antimicrobial activity.

Assessment of Probiotic Potential

Acid Resistance Testing

PBS buffer was prepared, and the pH was adjusted to 2.5, 3.5, and 7.4 using a pH meter. MRS broth was poured into Falcon tubes, inoculated with isolates, and incubated anaerobically at 37°C for 24 hours.

Assessment of Resistance to 0.3% Oxgall (Bile Salt)

For this test, 5.5 grams of MRS broth and 0.3 grams of bile salt (Oxgall) were accurately weighed using a digital balance and dissolved in distilled water to a final volume of 100 mL. The mixture was heated on a hot plate until completely dissolved. The medium was then sterilized by autoclaving at 121°C for 20 minutes. After cooling for 20 minutes, the culture media were aseptically transferred into Falcon tubes under a laminar flow hood and stored inverted in a refrigerator for 24 hours until use. Single colonies from isolates were inoculated into MRS broth tubes containing 0.3% bile salt and incubated anaerobically at 37°C for 24 hours. Control samples without bile salt were cultured under the same conditions.

The test tubes were monitored for bacterial growth or inhibition for up to five days post-inoculation.

Antioxidant Activity Using DPPH Assay

The antioxidant effect of bacterial strains on 2,2-diphenyl-1-picrylhydrazyl (DPPH) radicals was assessed using a modified method from Kim et al. and Lee et al. [7, 8] After culturing strains in MRS broth at 37°C for 18 hours, cells were washed twice with PBS, centrifuged at 4000 rpm for 5 minutes, and resuspended in PBS to an optical density at 600 nm (OD600) of 1.0. In the DPPH method, 0.2 mM DPPH solution and sample solution were mixed in a 1:2 (v/v) ratio and incubated at room temperature for 30 minutes. Absorbance was measured at 517 nm.[9]

Cell Culture

Hepatoblastoma (HepG2) cell lines were obtained from the Iranian Biological Resource Center and cultured according to standard protocols. Cells were maintained in Dulbecco's Modified Eagle Medium (DMEM) supplemented with 10% fetal bovine serum (FBS) and 1% penicillin-streptomycin at 37°C in a humidified atmosphere with 5% CO_2. Once cells reached 70% confluency, they were detached using 0.25% trypsin and centrifuged at 1500 rpm for 10 minutes. The resulting cell suspension was evaluated for viability using a light microscope.

Cytotoxicity Assay (MTT)

To assess the effect of LAB from camel milk on HepG2 cell proliferation, the 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay was employed. Cells were seeded into 96-well plates, and IC_{50} values were determined using MTT reagent (0.5 mg/mL, 20 µL per well). Plates were incubated for 4 hours, protected from light. Following incubation, the supernatant was removed, and 150 µL of dimethyl sulfoxide (DMSO) was added to dissolve formazan crystals. Within 20 minutes, the absorbance of the produced dye was measured using an ELISA reader at 570 nm. Cells were treated with the extract at various concentrations (3.125, 6.25, 12.5, 25, and 100 µg/mL of A4/A8) after 24 and 48 hours of incubation. Subsequently, the cells were cultured for an additional 3 hours at 37°C in an atmosphere containing 5% CO_2 and 95% O_2. Cell viability percentages were then calculated.

Caspase Activity

Caspase-3/7 activity was measured using the Kiazist kit (KCAS37, Iran) according to the manufacturer's protocol. Cells were treated for 48 hours with the IC_{50} concentrations of isolated extracts A4 and A8 (expressed in µg/mL). Samples were analyzed using a microplate reader at wavelengths of 400 or 405 nm. The increase in caspase-3/7 activity was determined by comparing the results with untreated control samples.[10]

RNA Extraction

Total RNA was extracted using the TRIzol method. Samples were homogenized and lysed in TRIzol reagent. After centrifugation, the aqueous RNA phase was separated and precipitated with chloroform and isopropanol. The RNA pellet was washed with 75% ethanol. RNA quantity and purity were assessed using a NanoDrop spectrophotometer and agarose gel electrophoresis. RNase-free materials and sterile conditions were maintained throughout the procedure.

cDNA Synthesis

Equal concentrations of RNA were prepared for cDNA synthesis. cDNA synthesis was performed using the RevertAid RT Transcription Kit (Thermo Fisher) according to the manufacturer's protocol. DNA must be synthesized from the RNA strand because DNA polymerase cannot use RNA as a template. As outlined in the table, RNA was converted into cDNA for Real-Time PCR using reverse transcriptase, aided by random hexamer primers. The synthesized cDNAs were stored at -70°C. Finally, the microtubes were placed in a thermal cycler following the program described in the table. The synthesized samples were subsequently stored at -20°C. (Table 1, Table 2)

Table 1 Components for cDNA synthesis

Component	Volume (µL)
Random hexamer	1
Total RNA	1
RNase-Free Water	10
5X Reaction Buffer	4
RiboLock RNase Inhibitor	1
dNTP Mix (10 mM)	2
RevertAid Reverse Transcriptase	1

Table 2 Thermal conditions for cDNA synthesis

Temperature (°C)	Time (min)
65	5
25	10
45	45
70	70

Primer Design

The Primer3 software was used for primer design. The target gene sequences were obtained from genomic databases. The following criteria will be considered for primer design: the melting temperature (Tm) of the primers should be between 58–60°C, with a maximum Tm difference of 1°C between forward and reverse primers. Primer lengths will be selected between 18 to

30 base pairs. The Tm of the primers should be between 58-60°C and the maximum difference in Tm of the primers should be 1°. The length of the primers should be between 18-30 bases. The percentage of CG of the primers should be close, ideally 50-60%. At least, one purine should be placed at the 3' end of the primer, but care should be taken that the 3' end of the primer is not rich in purines. The base T at the 3' end of the primer should be avoided, because thymine forms mismatches more easily than other bases. The ideal length of the amplicon is between 80-200 bases (Table 3).

Table 3 Primer specifications

Primer name		Sequence (5'-3')	Length	GC%	Tm (°C)
BAX	Forward	CCACCCTGGTCTTGGATCCAGCCC	24	66.67	68.71
	Reverse	CCTGTGCACCAAGGTGCCGGAACT	24	62.50	69.46
BCL2	Forward	TTGTGGCCTTCTTTGAGTTCGGTG	24	50.00	63.77
	Reverse	GGTGCCGGTTCAGGTACTCAGTCA	24	58.33	66.18
GAPDH	Forward	TGCCTCCTGCACCACCAAC	19	63.16	62.79
	Reverse	CGGAGGGGCCATCCACAG	18	72.22	62.18

Gene Expression Analysis by Real-Time PCR

One microgram of RNA was added to 1 microliter of 10x buffer and 1 microliter of DNAse and incubated for 30 minutes at 37°C. To inactivate DNAse, 1 µL of EDTA was added and incubated for 10 min at 65°C. Then, 4 µL of 5x buffer, 1.5 µL of MgCl2, 2 µL of dNTP mix (10 mM), 2 µL of random hexamer, 0.5 µL of RNasin (40 units/µL), and 1 µL of RT enzyme were added and the mixture was incubated for 60 min at 42°C. To inactivate RT, the samples were incubated for 10 min at 70°C and the products were stored at -20°C. micrograms (1 µg) of RNA was treated with DNase and incubated at 37°C for 30 minutes, followed by inactivation with 10 mM EDTA at 65°C for 10 minutes. The qPCR reaction (20 µL volume) included 2X SYBR Green Master Mix, specific primers (10 µM each), cDNA template, and nuclease-free water. The Exicycler 96 Real-Time PCR System was used with the thermal profile listed in Table 4. Gene expression was calculated using the 2^(-ΔΔCt) method, with GAPDH as the internal control gene.

Table 4 Primer specifications

Step	Time	Temp (°C)	Cycle
Initial denaturation	5 min	95	1
Denaturation	30 sec	95	40
Annealing extension	30 sec	60	

Data Analysis

Results are presented as mean ± SEM. Statistical analysis was performed using REST software, with $p < 0.0001$ considered statistically significant.

3 Results

Assessment of Probiotic Potential

Growth at Different pH Levels

This test was conducted on all eight isolates. Results showed that LAB from all isolates was capable of growing in environments with pH values of 2.5 and 3.5. Therefore, this test was deemed positive, indicating that all isolates possessed acid-resistance potential, a key probiotic characteristic.

Bile Salt Resistance (0.3% Oxgall)

The probiotic potential of the isolates was evaluated by assessing their tolerance to 0.3% bile salts. The results showed that all isolates were able to grow in the presence of bile salts, indicating their resistance under the test conditions.

Antioxidant Activity of Isolates A4 and A8

DPPH radical scavenging assay revealed dose-dependent antioxidant activity (Figure 1). Trolox (6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid) served as the standard, while green tea extract was the positive control. Isolate A4 showed 2–23% DPPH scavenging activity, whereas A8 exhibited 2–19% activity.

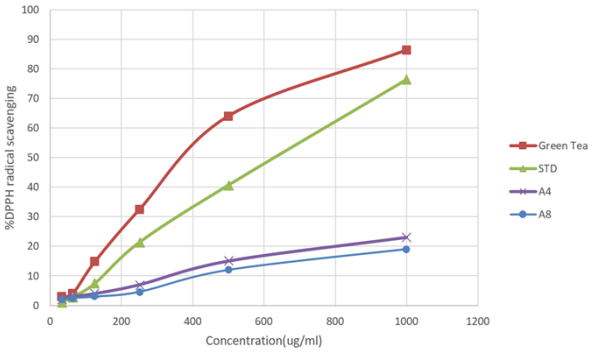

Figure 1 DPPH-based evaluation of antioxidant activity of isolates A4 and A8

Cytotoxicity Assessment of A4 and A8 Strains on the HepG2 Cell Line

HepG2 cells were treated with various concentrations

(3.125, 6.25, 12.5, 25, and 100 µg/mL) of isolates A4 and A8. Cell viability was assessed at 24 and 48 hours post-treatment.

For A4, at 24 hours and 570 nm absorbance, cell viability at 51.89 µg/mL showed a significant reduction compared to untreated controls (Figure 2). At 48 hours, the IC50 was determined to be 30.66 µg/mL, and the difference from the control was statistically significant. Concentrations higher than the IC50 showed pronounced cytotoxicity, indicating the isolate's toxic effect on cancer cells.

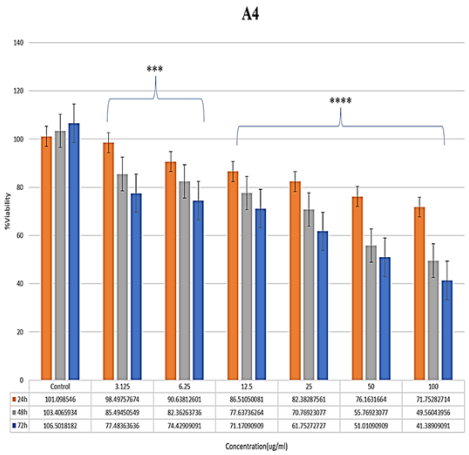

Figure 2 Effect of various concentrations of isolate A4 on HepG2 cell viability; $p < 0.0001$*** * , $p < 0.001$***

At 24 hours of treatment, absorbance measured at 570 nm for various concentrations of A8 showed a value of 67.29 µg/mL compared to the control group (untreated cells) (Figure 3). After 48 hours of treatment, the IC_{50} was determined to be 32.03 µg/mL, with a statistically significant difference in absorbance observed at this concentration compared to the control group (untreated). At concentrations higher than the IC_{50}, a pronounced decrease in HepG2 cell.

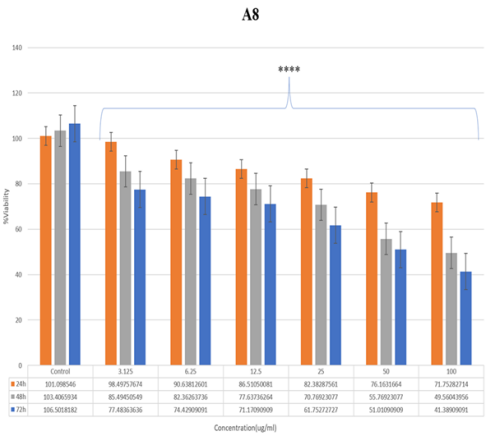

Figure 3 Effect of various concentrations of isolate A8 on HepG2 cell viability; $p < 0.0001$****

viability was observed, indicating cytotoxicity of these concentrations to cancer cells.

IC50 Values

The IC_{50} values for each compound at each time point were calculated using Prism software version 8 and are presented in Table 5.

Table 5 IC50 values

Step	Time	Temp (°C)	Cycle
Initial denaturation	5 min	95	1
Denaturation	30 sec	95	40
Annealing extension	30 sec	60	

Caspase 3/7 Activity

Caspases are enzymes that can be activated to disrupt cellular structures and induce apoptosis. Caspase activity was evaluated after HepG2 cells were treated with A4 and A8 isolates at their respective IC50 concentrations. Figure 4 shows that caspase activity increased in both treated groups compared to controls, with A4 demonstrating a higher effect than A8.

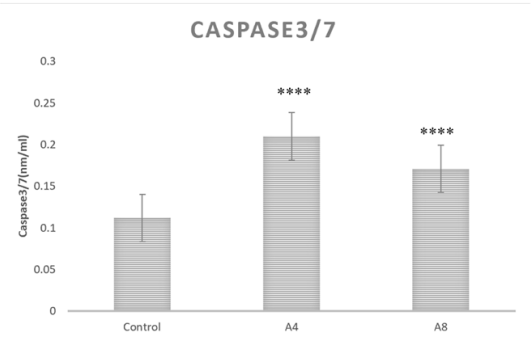

Figure 4 Caspase 3/7 activity in HepG2 cells treated with different samples. Data represent mean ± SD (n=3); $p < 0.001$****

Gene Expression Profiling of BAX and BCL-2 in HepG2 Cells Treated with Camel Milk using qRT-PCR

The expression of genes associated with apoptosis in liver cancer cells induced by camel milk was determined using the qRT-PCR method. The expression levels of the BAX and BCL2 genes in liver cancer cells were analyzed through Real-Time PCR. Amplification curves were generated by measuring changes in fluorescence intensity using a Real-Time PCR system, which reflected the amplification of the samples. Figure 5 illustrates the amplification curves for the GAPDH, BAX, and BCL2 genes. The expression of BCL2 was significantly decreased compared to GAPDH ($P = 0.0063$), whereas BAX expression increased compared to the control group; however, this increase was not statistically significant (P

= 0.4378) (Figure 5).

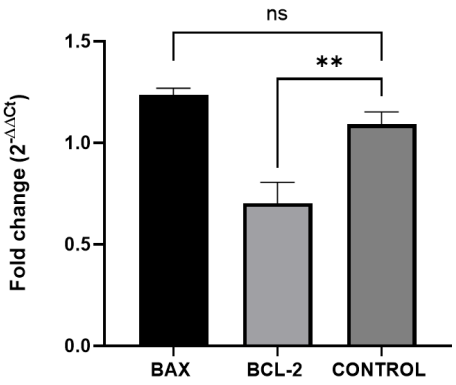

Figure 5 Expression of BAX and BCL-2 genes compared to the reference gene

4 Discussion

The aim of this study was to investigate the anticancer, pro-apoptotic (instead of anti-apoptotic, based on context) and antioxidant effects of LAB isolated from camel milk in the Mashhad region of Iran on liver cancer cells. Another key objective was to examine the effect of these bacteria on the expression of apoptosis-related genes, such as BAX and BCL-2, in liver cancer cells, thereby exploring potential mechanisms underlying their anticancer activity.

In this study, eight strains of Lactobacillus with probiotic properties were identified using phenotypic methods and biochemical tests. Among these, strains A4 and A8 exhibited the highest probiotic potential and were selected for further investigation. The DPPH radical scavenging activity ranged from 2% to 23% for strain A4 and from 2% to 19% for strain A8. Both strains demonstrated time- and dose-dependent cytotoxic effects on HepG2 liver cancer cells. The IC_{50} values at 48 h and 72 h for strain A4 were 89.51 µg and 66.30 µg, while for strain A8, the values were 29.67 µg and 3.32 µg (03.32 µg → corrected to 3.32 µg). Caspase activity increased in HepG2 cells following treatment with strains A4 and A8, with a greater increase observed for strain A4.[6]

Our findings are consistent with the study by Dharmisthaben et al., which assessed the anti-inflammatory and antioxidant properties of peptides released during the fermentation of camel milk by Lacticaseibacillus casei. That study used reversed-phase high-performance liquid chromatography (RP-HPLC) to isolate bioactive peptides based on molecular weight, particularly focusing on 3 kDa and 10 kDa (penetrating and inhibitory, respectively) fractions. The fermented camel milk was tested on LPS-stimulated RAW 264.7 macrophages and demonstrated both anti-inflammatory and antioxidant effects—similar to our findings—highlighting its role in mitigating oxidative stress associated with various diseases.[7]

Similarly, Orass T. Al-Hilift et al. explored the significance of camel whey proteins and their potential health benefits. Their study reported that hydrolyzed whey proteins effectively scavenged DPPH radicals and chelated iron ions, achieving 53.19% and 62.88% inhibition at 20 mg/mL, respectively. They also exhibited strong reducing power (77.199%), indicating substantial antioxidant potential. In comparison, the DPPH scavenging activity of our isolates A4 and A8 ranged from 2% to 23% and from 2% to 19%, respectively. We initially evaluated camel milk's ability to suppress HepG2 cell proliferation using the MTT assay, given its clinical relevance.[8]

Our results demonstrated that camel milk significantly inhibited the proliferation of HepG2 cells. A study by Korashy et al. also investigated the effects of camel milk on HepG2 and MCF-7 cancer cells, reporting an IC_{50} of approximately 76 mg/mL—higher than the values observed in our study.[9]

Our study specifically focused on the pro-apoptotic properties of LAB isolated from camel milk against liver cancer. The effects of LAB on cancer cell lines—particularly on the expression of BAX, BCL-2, and Caspase-3/7—have also been highlighted in recent studies. For example, Hemati et al. demonstrated that Lactobacillus sakei promoted apoptosis and reduced the proliferation of MCF-7 breast cancer cells by modulating the expression of BAX and BCL-2. Treatment with 5 mg/mL of the bacterial supernatant for 72 hours significantly increased BAX expression ($p = 0.0033$) and decreased BCL-2 expression ($p = 0.0278$).[10]

Likewise, the study by Korashy et al. identified activation of Caspase-3 and induction of death receptors in HepG2 and MCF-7 cells as potential mechanisms of camel milk's anticancer activity—findings consistent with ours.[9, 11]

The aim of this study was also to investigate the pro-apoptotic properties of LAB isolated from camel milk on liver cancer.

In 2022, studies focused on the anticancer potential of LAB strains from fermented foods, with their activity attributed mainly to the induction of apoptosis in cancer cells. However, due to the risk of sepsis—especially in immunocompromised patients—some studies employed heat-killed organisms. One such study using Lactobacillus brevis KU15176 showed that the strain upregulated apoptotic genes (BAX, Caspase-3, Caspase-9), triggered DNA fragmentation, and increased both apoptotic rates and caspase activity in AGS gastric adenocarcinoma cells, suggesting a preventive role for this strain.[12]

Conversely, another study reported that lactic acid, a tumor metabolite, decreases intracellular pH in NK cells, impairing mitochondrial function and inducing apoptosis—an effect that was mitigated by preventing

reactive oxygen species (ROS) accumulation.[13]

Another 2022 study isolated a probiotic LAB strain from camel milk with strong antioxidant activity against hydrogen peroxide, hydroxyl radicals, and superoxide anions. Identified as Lactobacillus helveticus E91 through 16S rRNA sequencing, its methanolic extract was separated by TLC and assessed for antioxidant activity using the DPPH method. The F4 fraction exhibited the highest DPPH scavenging and cytotoxic activity against Caco-2 cells, inducing 30.92% apoptosis as confirmed by flow cytometry. This study was the first to report the anticancer and pro-apoptotic effects of Lactobacillus helveticus against Caco-2 cells and recommended that this bioactive component be further investigated in human studies for cancer treatment.[14]

Another study examined three probiotic LAB strains isolated from human breast milk (Lactobacillus casei SR1, Lactobacillus casei SR2, and Lactobacillus paracasei SR4). These strains exhibited strong probiotic characteristics—including antibiotic sensitivity, antioxidant activity, and resistance to acid and bile. The cell-free culture supernatant (CFCS) of these strains also exhibited significant anticancer effects on cervical cancer cells (HeLa). Gene expression analysis revealed upregulation of BAX, BAD, Caspase-3, Caspase-8, and Caspase-9, alongside downregulation of BCL-2, supporting their potential therapeutic use.[15]

These findings suggest that Lactobacillus strains isolated from human breast milk may have therapeutic potential as a topical treatment against cervical cancer cells.[15]

5 Conclusion

The results of this study demonstrate the potential of LAB isolated from camel milk in Mashhad, Iran, as a promising agent for treating hepatocellular carcinoma cells. The anticancer, pro-apoptotic, and antioxidant effects of these bacteria were evident in both preclinical experiments and gene expression analyses. The observed modulation of BAX and BCL-2 gene expression suggests a novel mechanism for inhibiting cancer cell proliferation, providing a strong rationale for further exploration of these bacteria as a potential therapeutic option for liver cancer.

These findings contribute to the growing body of evidence supporting the use of probiotics in cancer prevention and treatment, and underscore the need for further research to elucidate the underlying mechanisms and optimize therapeutic strategies fully.

Declarations

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Authors' Contributions
All authors participated in the initial conceptualization, study design, data collection, and drafting of the manuscript. All authors have read and approved the final version and declare no conflicts regarding the content of this paper.

Availability of Data and Materials
The authors can make the contents of this article available to everyone upon request.

Conflict of Interest
The authors declare no conflicts of interest related to this study.

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The authors have full consent to publish this article.

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This research was derived from a thesis project approved with the Code of Ethics IR.IAU.PIAU.REC.1403.036. All ethical guidelines were observed, and the study was conducted at Islamic Azad University, Islamshahr Branch, Tehran.

Artificial Intelligence Disclosure
The authors declare that this manuscript was prepared without the use of AI tools.

References

1. Poua S, Mohammadi M. Evaluation of hybrid plasmonic nanocarriers and sea cucumber anticancer compounds in cancer treatment. Studies in Medical Sciences. 2025;35(12):994-1004.
2. Shaban AM, Raslan M, Sharawi ZW, Abdelhameed MS, Hammouda O, El-Masry HM, et al. Antibacterial, antifungal, and anticancer effects of camel milk exosomes: an in vitro study. Veterinary Sciences. 2023;10(2):124.
3. Behrouz S, Saadat S, Memarzia A, Sarir H, Folkerts G, Boskabady MH. The antioxidant, anti-inflammatory and immunomodulatory effects of camel milk. Frontiers in immunology. 2022;13:855342.
4. Al-Numair NS, Theyab A, Alzahrani F, Shams AM, Al-Anazi IO, Oyouni AAA, et al. Camels' biological fluids contained nanobodies: promising avenue in cancer therapy. Cancer Cell International. 2022;22(1):279.
5. Redwan ERM, Tabll A. Camel lactoferrin markedly inhibits hepatitis C virus genotype 4 infection of human peripheral blood leukocytes. Journal of immunoassay & immunochemistry. 2007;28(3):267-77.

6. Dharmisthaben P, Sakure A, Liu Z, Maurya R, Das S, Basaiawmoit B, et al. Identification and molecular mechanisms of novel antioxidative peptides from fermented camel milk (Kachchi breed, India) with anti-inflammatory activity in raw macrophages cell lines. International Journal of Dairy Technology. 2023;76(1):111-25.

7. Kim H, Kim J-S, Kim Y, Jeong Y, Kim J-E, Paek N-S, Kang C-H. Antioxidant and probiotic properties of Lactobacilli and Bifidobacteria of human origins. Biotechnology and Bioprocess Engineering. 2020;25(3):421-30.

8. Lee JY, Park JY, Park C-G, Kim DH, Ji Y-J, Choi SJ, et al. Validation of a method and evaluation of antioxidant activity for the simultaneous determination of riboflavin and coixol in Coix lacryma-jobi var. ma-yuen stapf sprouts. The Korean Journal of Crop Science. 2019;64(4):452~ 8-~ 8.

9. Schlesier K, Harwat M, Böhm V, Bitsch R. Assessment of antioxidant activity by using different in vitro methods. Free radical research. 2002;36(2):177-87.

10. Asghari Lalami Z, Tafvizi F, Naseh V, Salehipour M. Fabrication, optimization, and characterization of pH-responsive PEGylated nanoniosomes containing gingerol for enhanced treatment of breast cancer. Naunyn-Schmiedeberg's Archives of Pharmacology. 2023;396(12):3867-86.

11. Al-Hilifi SA, Al-Ibresam OT, Al-Hatim RR, Al-Ali RM, Maslekar N, Yao Y, Agarwal V. Development of chitosan/whey protein hydrolysate composite films for food packaging application. Journal of Composites Science. 2023;7(3):94.

12. Johnston JB, Kabore AF, Strutinsky J, Hu X, Paul JT, Kropp DM, et al. Role of the TRAIL/APO2-L death receptors in chlorambucil- and fludarabine-induced apoptosis in chronic lymphocytic leukemia. Oncogene. 2003;22(51):8356-69.

13. Korashy HM, Maayah ZH, Abd-Allah AR, El-Kadi AO, Alhaider AA. Camel milk triggers apoptotic signaling pathways in human hepatoma HepG2 and breast cancer MCF7 cell lines through transcriptional mechanism. BioMed Research International. 2012;2012(1):593195.

14. Hemati E, Sazegar H. Evaluation of Lactobacillus Sakei Probiotic Supernatant's Effectiveness in Promoting Apoptosis and Decreasing Breast Cancer MCF-7 Cancerous Cells through Modulation of Bax and Bcl2 Genes. Journal of Shahid Sadoughi University of Medical Sciences. 2024.

15. Abdalla K. An overview of the therapeutic effects of camel milk in the treatment of type 1 diabetes mellitus. Biomol Res Therap. 2014;3:118-24.

16. Hwang C-H, Lee N-K, Paik H-D. The anti-cancer potential of heat-killed Lactobacillus brevis KU15176 upon AGS cell lines through intrinsic apoptosis pathway. International Journal of Molecular Sciences. 2022;23(8):4073.

17. Harmon C, Robinson MW, Hand F, Almuaili D, Mentor K, Houlihan DD, et al. Lactate-mediated acidification of tumor microenvironment induces apoptosis of liver-resident NK cells in colorectal liver metastasis. Cancer immunology research. 2019;7(2):335-46.

18. Mirdamadi S, Mahta Mirzaei M, Akbari-Adergani B, Nasoohi N. Effect of pectin on anti-diabetic and anti-oxidant properties of fermented milk by lactic acid bacteria isolated from traditional Iranian dairy products. Journal of food science and technology (Iran). 2022;19(123):55-67.

19. Rajoka MSR, 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 & function. 2018;9(5):2705-15.