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ORIGINAL ARTICLE

Cytotoxic activity and apoptosis induction by supernatant of *Lentilactobacillus buchneri* on HT-29 colon cancer cells

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ABSTRACT

Background and Objectives: Colorectal cancer (CRC) is the fourth most commonly diagnosed cancer and the third most deadly cancer in the world. According to recent experimental reports, probiotics and their derivatives protect CRC patients from treatment-related side effects. Therefore, the present study aimed to investigate the cytotoxic impact of the cell-free supernatant (CFS) of *Lentilactobacillus buchneri* on the HT-29 cancer cell line.
Materials and Methods: In the current study, we used the *L. buchneri* CFS, which was well isolated and identified in our previous investigation from traditional yogurt in the Arak region of Iran. The apoptosis induction in HT-29 cancer cells was assessed by cell cytotoxicity, flow cytometry, and qRT-PCR.
Results: *L. buchneri* CFS inhibited the proliferation of HT-29 cancer cells in a time- and dose-dependent manner. The apoptotic effect of CFS was further supported by the flow cytometry data, which showed that the maximum incidence of apoptosis was observed in HT-29 cancer cells treated with the IC_{50} concentration of CFS after 72 hours. CFS of *L. buchneri* also exerted the up-regulating effect on the expression of pro-apoptotic genes including *BAX*, *CASP9*, and *CASP3*. *L. buchneri* CFS at an IC_{50} dose induced cell cycle arrest in the G0/G1 phase in HT-29 cells.
Conclusion: This study indicates that *L. buchneri* CFS can prevent colorectal cancer (CRC) development in patients by inducing cancer cell apoptosis. This finding suggests that the CFS of *L. buchneri* could be used as a therapeutic agent for the control of CRC.

Keywords: Colorectal cancer; Cell free-supernatant; Probiotic; *Lentilactobacillus buchneri*; Apoptosis; Cell cycle arrest

INTRODUCTION

In a healthy human microbiome, probiotics are essential bacteria (1). The two most common species of probiotics are *Lactobacillus* and *Bifidobacterium*. Lactic acid bacteria (LAB) have several health-promoting properties, including anti-tumor and anti-inflammatory capabilities, which are closely linked to the inhibition of allergic reactions (2, 3). The antioxidant activity of LAB strains has also been studied. Many species with reducing activity, reactive oxygen species scavenging abilities, and metal ion chelation have been found (4).

The incidence of colorectal cancer (CRC), now the third leading cause of cancer death worldwide, is increasing rapidly in emerging countries (5). By 2030, there are expected to be 1.1 million annual deaths and more than 2.2 million new cases of CRC worldwide (6). Mucosal epithelial cells often undergo non-cancerous proliferation before CRC develops.

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The CRCs that develop in the wall of the colon or rectum can puncture blood or lymphatic vessels, facilitating metastasis through the blood to local lymph nodes or distant organs. Several dietary and lifestyle choices can increase intestinal inflammation and alter the gut microbiota to induce an immune response, both of which may promote the formation of polyps and their development into cancer. Alterations in oncogenes and tumor suppressor genes may also confer a selective advantage to mucosal cells, promoting hyperproliferation and ultimately carcinogenesis (7). These mutations may be inherited or occur spontaneously. Despite increasing incidence, advances in CRC therapy have resulted in decreased CRC morbidity in second and third world countries (8).

Based on the characteristics of the tumor, there are reasonable choices for CRC treatment, such as surgery, radiation, targeted therapy, immunotherapy and chemotherapy. In order to increase the survival rate of CRC patients, chemotherapy is commonly used to achieve therapeutic goals. The inability of drugs to distinguish between cancer cells and healthy cells compromises the effectiveness of most existing treatments. This increases toxicity and has adverse effects on the human body (9). Probiotics have recently been used in the treatment of appropriate colorectal cancer patients undergoing chemotherapy to reduce postoperative gastrointestinal problems (10). LAB, a widespread group of Gram-positive microorganisms, are the most popular form of probiotics because of their beneficial effects on host health and their generally recognized as safe (GRAS) status (11). The anticancer properties of probiotics depend on their ability to inhibit tumor development and induce cell cycle arrest and death (12, 13). Despite this, probiotics have been shown to biotransform chemicals and anticancer drugs (14), altering drug bioavailability and therapeutic effects or even exacerbating some diseases (15). In this context, supplementation with bioactive compounds derived from probiotics, which have potent anti-inflammatory and antiproliferative activities, is currently being used as an innovative, individualized therapeutic strategy in the treatment of CRC (16). Short-chain fatty acids, bacteriocins, exopolysaccharides, nonribosomal lipopeptides, and other bacterial peptides are among the secretory metabolites recovered from probiotic cell-free supernatant (CFS) (17, 18).

It has been demonstrated that various species of *Bifidobacterium* and *Lactobacillus* CFS can either induce apoptosis or limit their proliferative ability in colon cancer cell lines (19). Chen et al (20). found that HT-29 cell membrane damage may be caused by high concentration of *Lactobacillus reuteri* BCRC14625 and *Lactobacillus johnsonii* BCRC17010 supernatants, which would enhance the release of lactate dehydrogenase. Recent research has revealed that postbiotic metabolites derived from *Lactobacillus plantarum* strains exhibit potent selective cytotoxicity, preserving normal cells while triggering apoptosis and anti-proliferative effects in HT-29 cells (21). To the best of our knowledge, *Lentilactobacillus buchneri* was one of the first probiotics to be studied in relation to cancer and is still used in research today. The effects of CFS produced by *L. buchneri* isolated from conventional dairy products, its cytotoxic effects and its ability to induce apoptosis on the HT-29 colorectal cancer cell line were the objectives of the current study.

MATERIALS AND METHODS

Isolation and characterization of bacterial strain. In the current study, we used the *L. buchneri* CFS that was well isolated and identified in our previous investigation from traditional yogurt in the Arak region of Iran (22). Bacterial isolates were grown in MRS broth and incubated at 37°C for 24, 48, and 72 hours. The optical density (OD) at 600 nm was used to identify the growth of the bacteria. The serial dilution method was used to calculate the number of viable bacterial cells on the MRS agar culture. The CFS obtained was stored at -20°C until the following tests were performed.

Cell culture and MTT assay. The cytotoxic effect of *L. buchneri* CFS was investigated on HT-29 (human cancer cell line, Pasteur Institute, Iran). Cancer cells were cultured in RPMI 1640 and DMEM (Gibco, Carlsbad, USA) medium supplemented with 10% FBS (Gibco, Carlsbad, USA), 100 u/mL penicillin (Gibco, Carlsbad, USA), and 100 g/mL streptomycin (Gibco, Carlsbad, USA) at 37°C in a humidified environment with 5% CO_2.

To investigate the cytotoxic activity of *L. buchneri* CFS on HT-29 cancer cells, we used the MTT assay. Briefly, after seeding and incubation of 10^4 cells/well in 96-well plates, the cells were treated with different concentrations of CFS (12.5, 25, 50, 100, and 200 µg/

mL) and incubated with MTT dye for 24, 48, and 72 hours. After the removal of the MTT reagent, DMSO was used to dissolve the formazan crystals formed by the live cells. An ELISA reader was used to measure the absorbance at 570 nm. The effects of CFS were demonstrated by IC_{50} values (22, 23).

The percentage of cell growth inhibition was determined in the following manner:

Cell Viability = Test OD/Control OD × 100

Apoptosis assay. The evaluation of apoptosis induction was conducted using the Annexin V-FITC kit (eBioscience, Affymetrix, USA) in accordance with the manufacturer's instructions. CFS IC_{50} values were applied to HT-29 cells (10^5 cells/well) in 6-well plates and the cells were then incubated for 24, 48, and 72 hours. A flow cytometer was used for cell analysis (Biocompare, USA). FlowJo software ver.7.6.5 was used to evaluate the data (22, 23).

Cell cycle assay. HT-29 cells were cultured for 24 hours in 6-well plates at a seeding density of 1×10^6 cells per well. Cancerous cells were treated at CFS IC_{50} levels and incubated for 24, 48, and 72 hours. Flow cytometry was used to measure cell cycle arrest in treated HT-29 cells as in the previous study. FlowJo software ver.7.6.5 was used to examine the data (24).

qRT-PCR procedure. The expression levels of mRNA for *BAX*, *BCL-2*, *CASP3*, *CASP9*, *CYCLIND1*, *MMP2*, and *VEGF* genes were measured in HT-29 cells exposed to the IC_{50} dose of CFS (Bioneer, Daejeon, Korea) using quantitative Real-Time PCR. The RNA extraction and cDNA synthesis procedures were the same as the protocol of our previous study. Next, using the following temperature protocol, the Real-Time PCR reaction was carried out on a light cycler (Bioneer, Daejeon, Korea): 95°C (1 minute); 95°C (15 seconds); 60°C (1 minute). The relative gene expression was determined using the REST 2009 software and the $2^{-\Delta\Delta Ct}$ method, assuming a perfect efficiency of the PCR (22).

Statistical analysis. Statistical analysis was performed using GraphPad Prism version 8. To evaluate statistical differences in the data, Student's t-test and/or one-way analysis of variance (ANOVA) were used. A difference was statistically significant when it was $P<0.05$.

RESULTS

Antiproliferative activity of *L. buchneri* CFS. The destructive effect of different concentrations of *L. buchneri* CFS was evaluated against HT-29 cancer cell line after 24, 48 and 72 hours of treatment (Fig. 1). According to Fig. 1A, each concentration of *L. buchneri* CFS can significantly reduce the survival rate of cancer cells after each single period compared to the control ($P<0.001$). However, the administration of 200 µg/mL *L. buchneri* CFS at 72 hours showed the strongest cytotoxic effect on cancer cells, reducing the viability of sixty-five percent of the cells. After 24, 48, and 72 hours, the IC_{50} concentrations of *L. buchneri* CFS were 1904, 937.6, and 538.7 µg/mL, respectively. We demonstrated that the IC_{50} of the 48- and 72-hour treatments were significantly reduced compared to the IC_{50} of the 24-hour treatments, and the IC_{50} of the 72-hour treatment also showed a remarkable reduction compared to both the 24- and 48-hour treatments ($P<0.001$, $P<0.0001$) (Fig. 1B).

***L. buchneri* CFS induced apoptosis activity.** Fig. 2 shows that when cells were labeled simultaneously with annexin V-FITC and PI, cells undergoing early apoptosis absorbed Annexin V-FITC, while necrotic cells received PI. The CFS of *L. buchneri* showed 11.76%, 17.17%, and 21.65% apoptotic cell death at 24, 48, and 72 hours, respectively, as depicted in Fig. 2. The proportion of necrotic cells in HT-29 cells treated with *L. buchneri* CFS was 2.79%, 1.04%, and 0.379% at 24, 48, and 72 hours, respectively. Apoptosis was found in HT-29 cells after *L. buchneri* CFS treatment at different time points compared to untreated cells. The cancerous cells exhibited a considerable increase in apoptosis at 24, 48, and 72 hours ($P<0.001$) in comparison to the untreated cells. However, HT-29 cells treated with probiotic *L. buchneri* CFS for 72 hours (compared to 24 and 48 hours) showed the highest increase in apoptotic rate ($P<0.01$). We also showed that the viability of cancer cells was dramatically reduced after 24, 48, and 72 hours of treatment with *L. buchneri* CFS ($P<0.001$).

Cell cycle arrest. *L. buchneri* CFS treatment induced the cell cycle arrest of HT-29 cells and inhibited their progression through the G0/G1 phase. In addition, the percentage of cells in sub-G1 and G1 phases was increased significantly compared to the other groups after 72 hours of *L. buchneri* CFS treatment ($P<0.05$ and

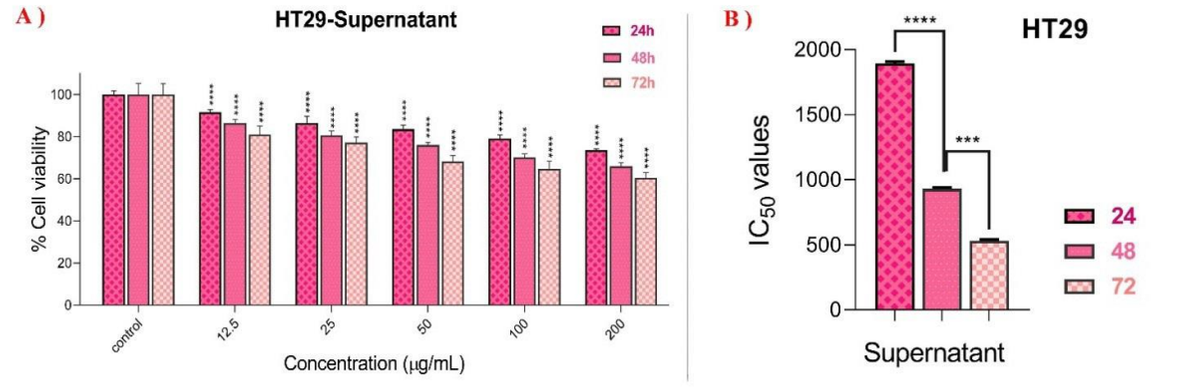

Fig. 1. (A) Cell viability of HT-29 cells after 24, 48, and 72 hours of treatment with CFS of *L. buchneri* (Results are presented as viability versus control). (B) A quantitative evaluation of CFS IC_{50} against HT-29 cancer cells (***P < 0.001, ****P < 0.0001).

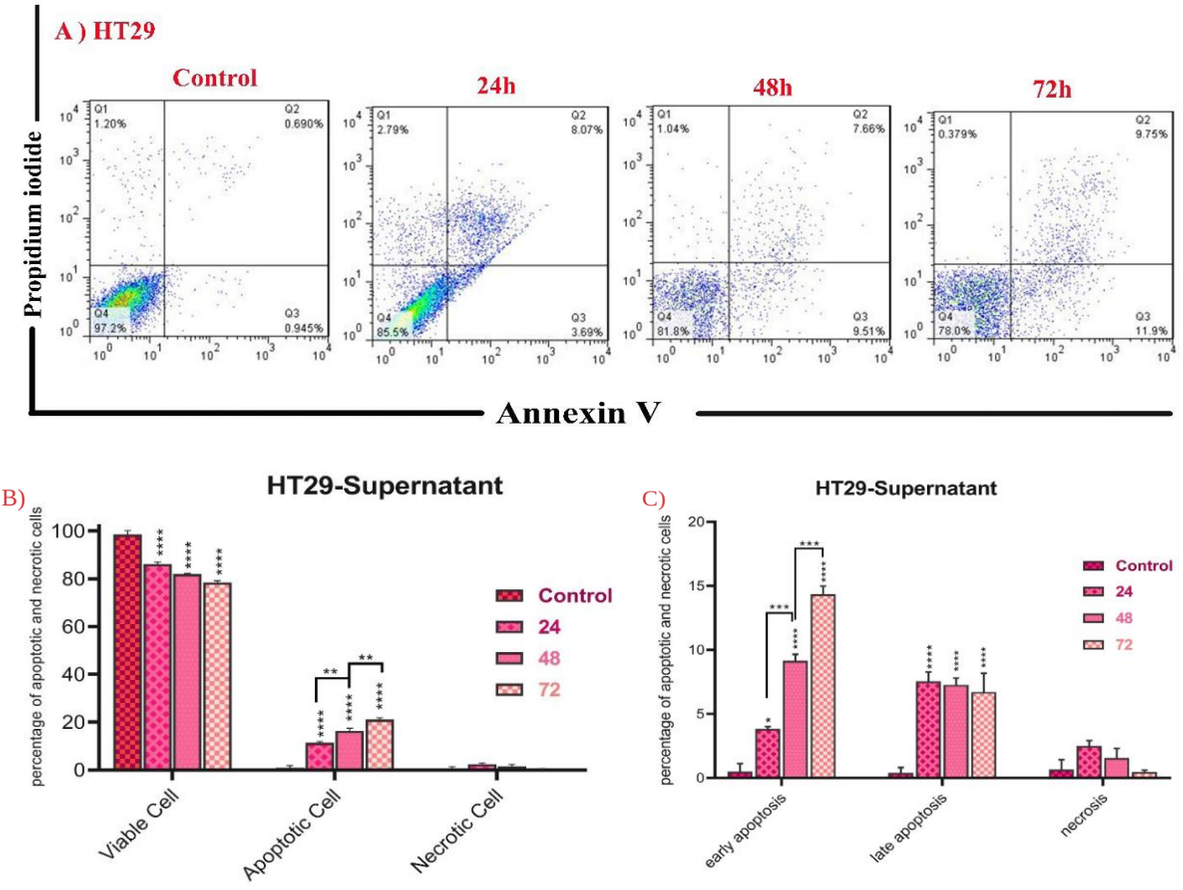

Fig. 2. (A) Diagram of flow cytometry to evaluate apoptosis induced by *L. buchneri* CFS at 24, 48, and 72 hours in HT-29 cells vs. control cells (untreated cancer cells); (B), (C) Percentage of apoptotic and necrotic cells in treated cells at 24, 48, and 72 hours. (***P< 0.001 and ****P < 0.0001).

P<0.01, respectively). The analysis of the cell cycle revealed that 65% of the cancer cells were arrested in the G1 phase. In addition, there was a significant reduction in the number of cells in the S phase at 24 and 72 hours (P<0.05 and P<0.001, respectively) (Fig. 3).

Gene expression level. The data in Fig. 4 displays the gene expression levels in HT-29 cells treated with

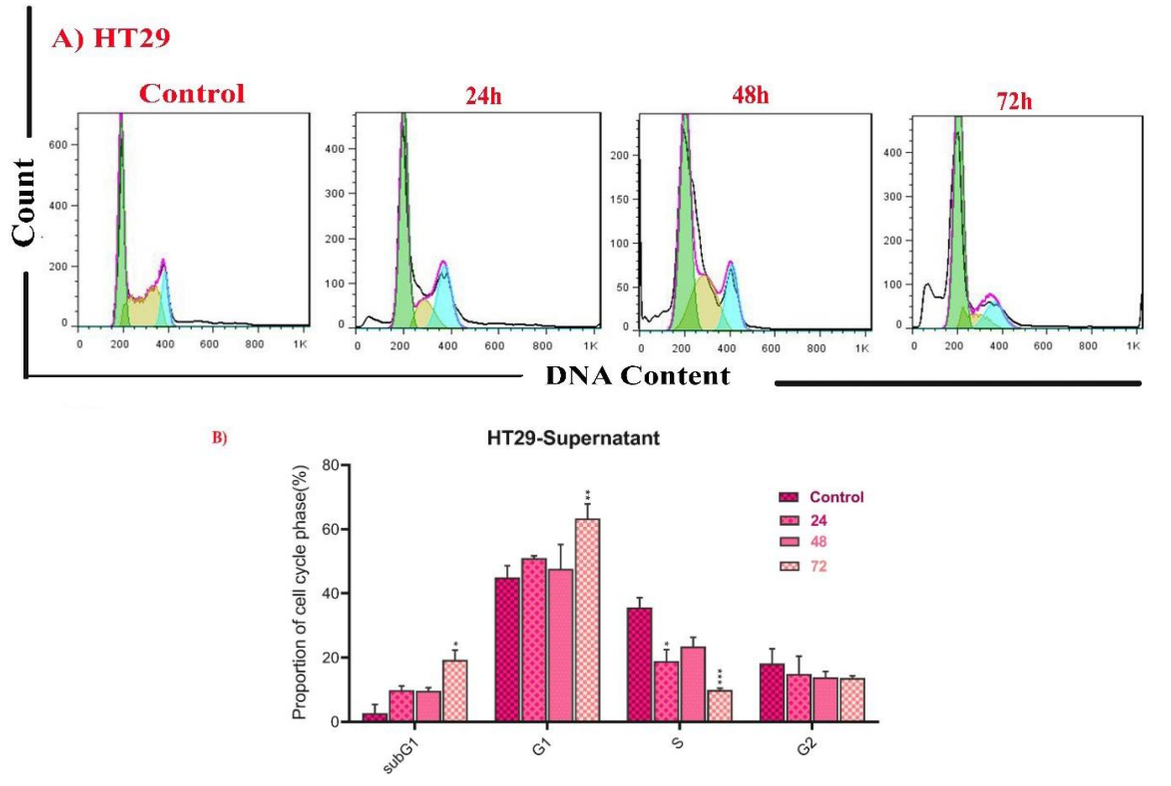

Fig. 3. (A) Diagram of flow cytometry for cell cycle arrest in HT-29 cells treated with *L. buchneri* CFS for 24, 48, and 72 hours vs. control cells (untreated HT-29 cells); (B) Proportion of cell cycle phase in treated cells after 24, 48, and 72 hours. (*P<0.05, **P<0.0, and ***P < 0.001).

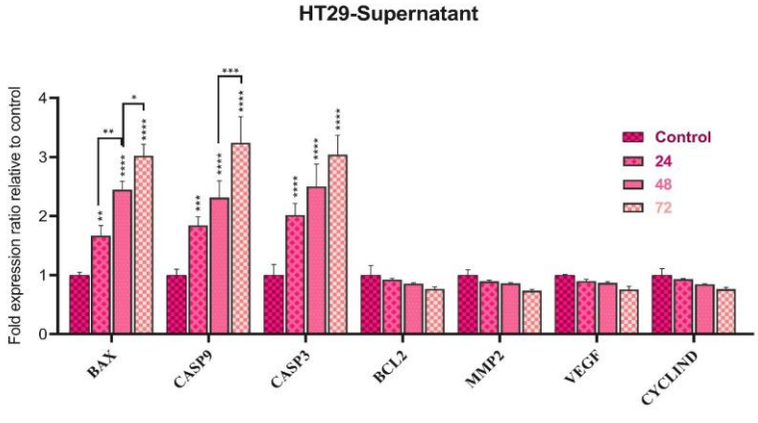

Fig. 4. The expression levels of target genes in HT-29 cell line treated with *L. buchneri* CFS for 24, 48, and 72 hours compared to control gene. (*P<0.05, **P<0.01, ***P < 0.001, and ****P < 0.0001).

L. buchneri CFS at the IC_{50} concentration over 24, 48, and 72 hours. Three categories of genes can be distinguished among them: angiogenic, pro-apoptotic, and anti-apoptotic. The findings demonstrated that during 24, 48, and 72 hours of treatment, the expression of the *BAX*, *CASP9*, and *CASP3* genes was significantly higher than that of the control group (P<0.001, P<0.0001). Particularly, *BAX* and *CASP9* expression significantly increased after 72 hours compared to earlier time points (P<0.05, P<0.001), suggesting a time-dependent anticarcinogenic impact of *L. buchneri* CFS. No significant differences were observed in the expression of *BCL2*, *MMP2*, *VEGF*, and *CCND1* genes between the groups.

DISCUSSION

CRC is a significant global cause of mortality. Because traditional cancer treatment is sometimes ineffective, there is increasing interest in new "drug-free" cancer therapies or interventions that enhance the beneficial effects of current therapy (25). Probiotics have an extensive record of safe use as preventive and adjunctive medications in the treatment of human disease. Probiotic bacteria may benefit health by producing anticarcinogenic, anti-inflammatory, antimutagenic, and other physiologically important chemicals (26, 27). The origin and probiotic efficacy of lactobacilli species are strongly influenced by their environment; therefore, the source from which they originate is critical. This study aimed to introduce a potent probiotic strain capable of treating cancer without the need for invasive methods (28). However, a crucial limitation of the current study is that the basis of our investigation is based on *in vitro* observations, which is also felt the need for *in vivo* studies.

L. buchneri was shown to be effective in suppressing proliferation and inducing apoptosis in HT-29 cells. The duration and dosage of probiotic bacterial supernatant affected the ability to inhibit proliferation and induce cell death. Compared to the control group, apoptosis was induced after 24, 48, and 72 hours of CFS therapy, with the 72-hour treatment having the highest apoptotic effect on HT-29 cancer cells. Cytotoxicity research focuses on the anticancer and apoptotic properties of *Lactobacillus* in various cell lines *in vitro* (29). *L. rhamnosus* GG is capable of preventing CRC, promoting apoptosis and reducing inflammation (30). The research examined the detrimental effect of probiotic *Lactobacillus* and CFS on the development of HT-29 cells, which found that *Lactobacillus* had different apoptotic effects on HT-29 cells (20). The overall survival of CRC *in vitro* was reduced to 78% when *L. casei* ATCC 393 at 10^9 CFU/mL was cultured with HT-29 cells for 24 hours (31). Guo et al. discovered that *L. acidophilus* CICC 6074 suppressed cell growth in a manner dependent on both time and dosage (32). This was consistent with our scientific results. The other research discovered that *L. plantarum* 06CC2 extract inhibited the development of Caco-2 cells *in vitro*. The *Lactobacillus* extract was found to inhibit cancer growth (33). The use of various concentrations of *L. acidophilus* extracts to inhibit cell growth in colorectal cancer has been documented. It was also indicated that CFS of some probiotics had an anti-cancer activity against AGS, HT-29, LoVo, MDA-MB-231, MCF-7, Hela, and SK-MES-1 cells (34-36). Our previous study showed that CFS of *L. buchneri* induced anti-proliferative effects against AGS cancer cells, and the calculated IC_{50} was almost close to the IC_{50} obtained in this study against HT-29 cancer cells (22).

Consistent with the cell cytotoxicity study, flow cytometry results showed that *L. buchneri* CFS could induce apoptosis in HT-29 cancer cell lines in a time-dependent manner. These results were also confirmed by evaluating the expression of apoptotic genes. Pro-apoptotic genes such as *BAX, CASP9* and *CASP3* were also upregulated. The increased expression of these genes may have stimulated the intrinsic mitochondrial pathway and triggered apoptosis. However, there were no remarkable changes in the expression of anti-apoptotic *BCL2* gene, metastatic *MMP2* gene, angiogenic *VEGF* gene. In addition, cell cycle studies revealed that *L. buchneri* CFS may increase cell arrest in the sub-G1 stage of the cell cycle and decrease cell population in the S phase after 72 hours of treatment, indicating inhibition of cell proliferation. Although, the qRT-PCR results didn't show significant decrease in the expression of cell cycle progression genes (cyclin D1or *CCND1*), which indicates that *L. buchneri* CFS may be involved in cell death an intrinsic pathway and has no effect on the expression of metastatic and angiogenic genes.

Growth factor deprivation and intracellular stress can induce intrinsic cell death, whereas transmembrane death receptors induce extrinsic apoptosis. The BCL-2 and caspase protein families regulate the initiation and execution of processes. BAX upregulation leads to outer mitochondrial membrane permeabilization, releasing cytochrome c into the cytosol. Cytochrome c binds to Apaf-1, forming an apoptosome and activating caspase-9. Activated caspase-9 then cleaves and activates caspase-3 and caspase-7. Caspase, a cytoplasmic protease, is preferentially activated in apoptosis, playing a crucial role in this cellular process (37).

In apoptosis, caspase-3 cleaves and activates caspases-6, -7, and -9, which lead to the degradation of apoptotic cells. However, ROS generation can be enhanced when caspase-9 blocks cytochrome C access to complex III in the mitochondria (38). Consistent with our findings, *L. casei* SR1, SR2, and *L. paracasei* SR4 exert anticancer effects against cervical cancer cells through the intrinsic mitochondri-

al apoptotic pathway, resulting in the stimulation of apoptosis. The researchers discovered that the activation of apoptosis was carried out by the upregulation of *BAD, BAX, CASP3, CASP8, CASP9* and a reduction of *BCL2* genes in HeLa cells treated with *L. buchneri* CFS (35). Our recent study on AGS cells showed an increased number of cells in sub-G1 phase (arrest in G0/G1 phase) and increased mRNA expression of *BAX, CASP9,* and *CASP3* after treatment with *L. buchneri* CFS (22). Therefore, we hypothesized that *L. buchneri* CFS may induce apoptosis by permeabilizing the mitochondrial outer membrane and increasing the expression of pro-apoptotic genes.

CONCLUSION

In conclusion, the *L. buchneri* CFS exhibited outstanding anticancer efficacy against the HT-29 cancer cells. Increased expression of *BAX, CASP3,* and *CASP9* resulted in anticancer efficacy. This study showed that *L. buchneri* from traditional Iranian yogurt can protect against CRC by inducing apoptosis in cancer cells. Furthermore, all these important biological activities may be useful for future *in vivo* investigations in animal models to illustrate the potential relevance of such *Lactobacillus* strains as organic drugs for both prevention and cure of CRC. Our data suggest that *L. buchneri* CFS inhibits HT-29 cell proliferation in a time- and concentration-dependent manner, possibly by inducing HT-29 cell death through an intrinsic mitochondrial apoptotic pathway and causing cancer cell cycle arrest.

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REFERENCES

1. Roy S, Trinchieri G. Microbiota: a key orchestrator of cancer therapy. *Nat Rev Cancer* 2017; 17: 271-285.
2. Ding C, Tang W, Fan X, Wu G. Intestinal microbiota: a novel perspective in colorectal cancer biotherapeutics. *Onco Targets Ther* 2018; 11: 4797-4810.
3. Lee J-E, Lee J, Kim JH, Cho N, Lee SH, Park SB, et al. Characterization of the anti-cancer activity of the probiotic bacterium *Lactobacillus fermentum* using 2D vs. 3D culture in colorectal cancer cells. *Biomolecules* 2019; 9: 557.
4. Sullivan Å, Nord CE, Evengård B. Effect of supplement with lactic-acid producing bacteria on fatigue and physical activity in patients with chronic fatigue syndrome. *Nutr J* 2009; 8: 4.
5. Soerjomataram I, Bray F. Planning for tomorrow: global cancer incidence and the role of prevention 2020–2070. *Nat Rev Clin Oncol* 2021; 18: 663-672.
6. Arnold M, Sierra MS, Laversanne M, Soerjomataram I, Jemal A, Bray F. Global patterns and trends in colorectal cancer incidence and mortality. *Gut* 2017; 66: 683-691.
7. Rawla P, Sunkara T, Barsouk A. Epidemiology of colorectal cancer: incidence, mortality, survival, and risk factors. *Prz Gastroenterol* 2019; 14: 89-103.
8. Edwards BK, Ward E, Kohler BA, Eheman C, Zauber AG, Anderson RN, et al. Annual report to the nation on the status of cancer, 1975-2006, featuring colorectal cancer trends and impact of interventions (risk factors, screening, and treatment) to reduce future rates. *Cancer* 2010; 116: 544-573.
9. Tanis PJ, Tuynman JB. Long-term safety of laparoscopic rectal cancer resection. *Lancet Gastroenterol Hepatol* 2021; 6: 516-518.
10. Huang F, Li S, Chen W, Han Y, Yao Y, Yang L, et al. Postoperative probiotics administration attenuates gastrointestinal complications and gut microbiota dysbiosis caused by chemotherapy in colorectal cancer patients. *Nutrients* 2023; 15: 356.
11. Garbacz K. Anticancer activity of lactic acid bacteria. *Semin Cancer Biol* 2022; 86: 356-366.
12. Lu K, Dong S, Wu X, Jin R, Chen H. Probiotics in cancer. *Front Oncol* 2021; 11: 638148.
13. Dicks LM, Vermeulen W. Do bacteria provide an alternative to cancer treatment and what role does lactic acid bacteria play? *Microorganisms* 2022; 10: 1733.
14. Mavromatis P, Stampouli K, Vliora A, Mayilyan A, Samanidou V, Touraki M. Development of an HPLC-DAD method for the extraction and quantification of 5-Fluorouracil, Uracil, and 5-Fluorodeoxyuridin Monophosphate in cells and culture media of lactococcus lactis. *Separations* 2022; 9: 376.
15. Purdel C, Ungurianu A, Adam-Dima I, Margină D. Exploring the potential impact of probiotic use on drug metabolism and efficacy. *Biomed Pharmacother* 2023; 161: 114468.
16. Song D, Wang X, Ma Y, Liu N-N, Wang H. Beneficial insights into postbiotics against colorectal cancer. *Front Nutr* 2023; 10: 1111872.
17. Thoda C, Touraki M. Probiotic-derived bioactive compounds in colorectal cancer treatment. *Microorganisms* 2023; 11: 1898.

18. Mojibi P, Tafvizi F, Bikhof Torbati M. Cell-bound exopolysaccharide extract from indigenous probiotic bacteria induce apoptosis in HT–29 cell-line. *Iran J Pathol* 2019; 14: 41-51.
19. Escamilla J, Lane MA, Maitin V. Cell-free supernatants from probiotic *Lactobacillus casei* and *Lactobacillus rhamnosus* GG decrease colon cancer cell invasion *in vitro*. *Nutr Cancer* 2012; 64: 871-878.
20. Chen Z-Y, Hsieh Y-M, Huang C-C, Tsai C-C. Inhibitory effects of probiotic *Lactobacillus* on the growth of human colonic carcinoma cell line HT-29. *Molecules* 2017; 22: 107.
21. Chuah L-O, Foo HL, Loh TC, Mohammed Alitheen NB, Yeap SK, Abdul Mutalib NE, et al. Postbiotic metabolites produced by *Lactobacillus plantarum* strains exert selective cytotoxicity effects on cancer cells. *BMC Complement Altern Med* 2019; 19: 114.
22. Abedi A, Tafvizi F, Akbari N, Jafari P. Cell-Free Supernatant of *L. buchneri* probiotic bacteria enhancing apoptosis activity in AGS gastric cancer cells. *Iran J Sci* 2023; 47: 1071-1079.
23. Dolati M, Tafvizi F, Salehipour M, Movahed TK, Jafari P. Inhibitory effects of probiotic *Bacillus coagulans* against MCF7 breast cancer cells. *Iran J Microbiol* 2021; 13: 839-847.
24. Dolati M, Tafvizi F, Salehipour M, Komeili Movahed T, Jafari P. Antiproliferative potential of *Bacillus coagulans* supernatant on SKBR3 breast cancer cell line. *Iran J Med Microbiol* 2022; 16: 383-391.
25. Kvakova M, Kamlarova A, Stofilova J, Benetinova V, Bertkova I. Probiotics and postbiotics in colorectal cancer: Prevention and complementary therapy. *World J Gastroenterol* 2022; 28: 3370-3382.
26. Fong W, Li Q, Yu J. Gut microbiota modulation: a novel strategy for prevention and treatment of colorectal cancer. *Oncogene* 2020; 39: 4925-4943.
27. Kvakova M, Bertkova I, Stofilova J, Savidge TC. Co-encapsulated synbiotics and immobilized probiotics in human health and gut Microbiota modulation. *Foods* 2021; 10: 1297.
28. Cano-Garrido O, Seras-Franzoso J, Garcia-Fruitós E. Lactic acid bacteria: reviewing the potential of a promising delivery live vector for biomedical purposes. *Microb Cell Fact* 2015; 14: 137.
29. Orlando A, Refolo M, Messa C, Amati L, Lavermicocca P, Guerra V, et al. Antiproliferative and proapoptotic effects of viable or heat-killed *Lactobacillus paracasei* IMPC2. 1 and *Lactobacillus rhamnosus* GG in HGC-27 gastric and DLD-1 colon cell lines. *Nutr Cancer* 2012; 64: 1103-1111.
30. Gamallat Y, Meyiah A, Kuugbee ED, Hago AM, Chiwala G, Awadasseid A, et al. *Lactobacillus rhamnosus* induced epithelial cell apoptosis, ameliorates inflammation and prevents colon cancer development in an animal model. *Biomed Pharmacother* 2016; 83: 536-541.
31. Tiptiri-Kourpeti A, Spyridopoulou K, Santarmaki V, Aindelis G, Tompoulidou E, Lamprianidou EE, et al. *Lactobacillus casei* exerts anti-proliferative effects accompanied by apoptotic cell death and up-regulation of TRAIL in colon carcinoma cells. *PLoS One* 2016; 11(2): e0147960.
32. Guo Y, Zhang T, Gao J, Jiang X, Tao M, Zeng X, et al. Lactobacillus acidophilus CICC 6074 inhibits growth and induces apoptosis in colorectal cancer cells *in vitro* and in HT-29 cells induced-mouse model. *J Funct Foods* 2020; 75: 104290.
33. Hiraishi N, Kanmura S, Oda K, Arima S, Kumagai K, Mawatari S, et al. Extract of *Lactobacillus plantarum* strain 06CC2 induces JNK/p38 MAPK pathway-mediated apoptosis through endoplasmic reticulum stress in Caco2 colorectal cancer cells. *Biochem Biophys Rep* 2019; 20: 100691.
34. Azam R, Ghafouri-Fard S, Tabrizi M, Modarressi M-H, Ebrahimzadeh-Vesal R, Daneshvar M, et al. *Lactobacillus acidophilus* and *Lactobacillus crispatus* culture supernatants downregulate expression of cancer-testis genes in the MDA-MB-231 cell line. *Asian Pac J Cancer Prev* 2014; 15: 4255-4259.
35. Riaz Rajoka MS, Zhao H, Mehwish HM, Li N, Lu Y, Lian Z, et al. Anti-tumor potential of cell free culture supernatant of *Lactobacillus rhamnosus* strains isolated from human breast milk. *Food Res Int* 2019; 123: 286-297.
36. Abdoli M, Fathi Rezaei P, Mansouri K. Exploring the anticancer efficacy of mixture of local probiotics on MDA-MB-231 and MCF-7 breast cell lines. *J Cell Mol Res* 2021; 13: 54-64.
37. Fan T-J, Han L-H, Cong R-S, Liang J. Caspase family proteases and apoptosis. *Acta Biochim Biophys Sin (Shanghai)* 2005; 37: 719-727.
38. Brentnall M, Rodriguez-Menocal L, De Guevara RL, Cepero E, Boise LH. Caspase-9, caspase-3 and caspase-7 have distinct roles during intrinsic apoptosis. *BMC Cell Biol* 2013; 14: 32.