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ORIGINAL ARTICLE / ÖZGÜN MAKALE

Can buttermilk (ayran) with its postbiotic content be used in the protection of colon health?

Postbiyotik içeriğiyle ayran kolon sağlığının korunmasında kullanılabilir mi?

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Abstract

Objective: In recent years, we have come across articles on the positive effects of nutrition in disease prevention and treatment processes. The microbiota formed by bacteria in the human body can play a role in various diseases and cancer. There is some information on the prevention and treatment of colon cancer by products called postbiotics produced by some bacteria in this flora. It was aimed to investigate the therapeutic effects of ayran, an ingredient rich in postbiotic products, on colon cancer.
Materials and Methods: This study evaluates the effects of postbiotic LTW 35 on normal colon fibroblast (CRL-1459) and colon cancer (CCL-224) cell lines. CRL-1459 cells treated with TT X100 for cytotoxicity and CCL-224 cells grown to sufficient density were exposed to normal buttermilk and buttermilk containing 1%, 2%, 3%, and 4% postbiotic LTW 35. Cell viability was assessed using the MTT assay, and tumor activity was measured via the Ca 19-9 tumor marker.
Results: The viability of CRL-1459 colon fibroblast cells decreases progressively with increasing concentrations of TT X100, reaching its lowest level at 0.5%. The viability of colorectal cancer cells is reduced as the concentration of postbiotic LTW 35 (*Streptococcus thermophilus* ATA-LTC St140700, *Bifidobacterium animalis* ATA-BSLA0310, *Lactobacillus acidophilus* ATA-LAP1201 ferment extract lysate) increases, with the lowest viability observed at 4%. Ca19-9 tumor marker levels in cancer cells decrease gradually with increasing concentrations of postbiotic LTW 35, showing the most significant reduction at 4%.
Conclusion: Postbiotic LTW 35-enriched buttermilk restores the viability of TTX 100-damaged normal colon fibroblast cells and reduces the viability of colorectal cancer cells in a concentration-dependent manner, indicating both restorative and anticancer effects. The observed decrease in Ca19-9 tumor marker levels further highlights its potential in reducing tumor activity.

Keywords: Cell Viability, Buttermilk (Ayran), Colon Cancer, Postbiotics

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Öz

Amaç: Son yıllarda hastalıklardan korunma ve tedavi süreçlerinde beslenmenin olumlu etkilerine ilişkin makalelere rastlıyoruz. İnsan vücudundaki bakterilerin oluşturduğu mikrobiyota çeşitli hastalıklarda ve kanserde rol oynayabiliyor. Bu florada yer alan bazı bakterilerin ürettiği postbiyotik adı verilen ürünlerle kolon kanserinin önlenmesi ve tedavisi konusunda bazı bilgiler mevcuttur. Bu çalışmada, postbiyotik ürünler açısından zengin bir bileşen olan ayranın kolon kanseri üzerindeki tedavi edici etkilerinin araştırılması amaçlanmıştır.
Gereç ve Yöntem: Bu çalışmada, postbiyotik LTW 35'in normal kolon fibroblast (CRL-1459) ve kolon kanseri (CCL-224) hücre hatları üzerindeki etkileri değerlendirilmiştir. Sitotoksisite için TT X100 ile muamele edilen CRL-1459 hücreleri ve yeterli yoğunlukta büyütülen CCL-224 hücreleri normal ayrana ve %1, %2, %3 ve %4 postbiyotik LTW 35 içeren ayrana maruz bırakılmıştır. Hücre canlılığı MTT testi kullanılarak değerlendirilmiş ve tümör aktivitesi Ca 19-9 tümör belirteci ile ölçülmüştür.
Bulgular: CRL-1459 kolon fibroblast hücrelerinin canlılığı, artan TT X100 konsantrasyonları ile düzenli olarak azalmış ve %0,5'te en düşük seviyeye ulaşmaktadır. Kolorektal kanser hücrelerinin canlılığı, postbiyotik LTW 35 (*Streptococcus thermophilus* ATA-LTC St140700, *Bifidobacterium animalis* ATA-BSLA0310, *Lactobacillus acidophilus* ATA-LAP1201 ferment ekstrakt lizatı) konsantrasyonu arttıkça azalmakta ve en düşük canlılık %4'te gözlenmektedir. Kanser hücrelerindeki Ca19-9 tümör işaretleyici seviyeleri, artan postbiyotik LTW 35 konsantrasyonları ile kademeli olarak azalmakta ve en önemli azalma %4'te görülmektedir.
Sonuç: Postbiyotik LTW 35 ile zenginleştirilmiş ayran, TTX 100 ile hasar görmüş normal kolon fibroblast hücrelerinin canlılığını geri kazandırmakta ve kolorektal kanser hücrelerinin canlılığını konsantrasyona bağlı bir şekilde azaltarak hem onarıcı hem de antikanser etkilerini göstermektedir. Ca19-9 tümör marker seviyelerinde gözlenen düşüş, tümör aktivitesini azaltma potansiyelini daha da vurgulamaktadır.

Anahtar Kelimeler: Hücre Canlılığı, Ayran, Kolon Kanseri, Postbiyotikler

INTRODUCTION

Colon cancer is a highly prevalent form of human malignant tumor, with approximately 1,148,000 new cases and 576,000 new deaths reported globally in 2020 (1). Today, surgery remains the primary treatment for patients with early-stage colon cancer. However, most colon cancer patients are diagnosed at advanced stages (2). Despite significant advances in combined treatment strategies for colon cancer, patients often face low 5-year survival rates (3). Advances in screening, surgical techniques, and adjuvant therapies led to substantial improvement in outcomes of patients diagnosed with colon cancer (4,5).

The human body harbors an estimated three trillion bacterial members that orchestrate a comprehensive interplay of physiological processes and disease susceptibilities (6). The surface of the human colonic epithelium is exposed to and interacts with highly complex and metabolically sophisticated bacterial ecosystems. The gut microbiota has an important role in maintaining a healthy human colon by protecting the host against pathogen overgrowth, shaping the development of innate and adaptive immunity, and obtaining energy and producing nutrients for the host (7). The human microbiome forms a complex multikingdom community that interacts symbiotically with its host, the human, at various sites in the body. Host-microbiome interactions influence multiple physiological processes and various multifactorial disease states (8).

The presence of abnormal changes in the intestinal microflora of colon cancer patients suggests that disruption of the intestinal microflora is closely associated with the initiation and progression of colon cancer cells (9, 10). Furthermore, probiotics have been shown to have tumor suppressive effects in colon cancer cell lines and mouse/rat tumor models (11, 12). The microbiota has been shown to produce small molecules and metabolites that have both local and systemic effects on cancer initiation, progression and treatment response (13).

Probiotics, microbiota and colon health

The gut microbiota has broad influences that contribute to the host immune system during tumor formation (14,15). The relationships between the gastrointestinal microbiota and systemic lymphoid tissues have increased interest in microbial modulation as a powerful immunotherapeutic modality. for example, modulation of the gut microbiota influences the composition of the intratumoral microbiome in pancreatic cancer, possibly through pancreatic duct communication (16-18) and modulation of gut microbiota aimed at restoring gut microbial homeostasis is becoming a potential strategy for the prevention and treatment of colon cancer (19).

Probiotics are associated with a variety of health benefits, including improving gut microflora, suppressing extreme allergic responses and tumor suppressive effects (20-22) and probiotics are defined as live microorganisms that, when administered in adequate amounts, provide health benefits to the host (23). Probiotics are now recognized to function beyond mediating the microbiota, but also to cause physiological and metabolic changes in the host (19).

Intestinal health is impaired for various reasons. Small intestinal bacterial overgrowth (SIBO), is a sickness and characterized by excessive bacterial colonization in the small intestine. There is a study on rats using probiotics by Aslan et al. and in this study he observed the important role of probiotics in the amelioration of small intestinal bacterial overgrowth (SIBO) (24). Younesi and Ayseli, in a study, stated that there is an urgent need for innovative models to strengthen functional food production processes (25). Ayseli et al. stated that the results obtained from their study constituted an important first step towards fermented foods and their health effects on various infections (26). Another important food group containing a mixture of probiotic bacteria is milk and dairy products. In a study by Bursalioglu examining the effects of human milk, mare's milk and cow colostrum milk on A549 lung cancer cell lines; the possibility that human milk may have a therapeutic role in lung cancer treatment. (27).

Postbiotics, microbiota and colon health

Postbiotic term is clearly articulated as any factor resulting from the metabolic activity of a probiotic or any released molecule that can provide beneficial effects to the host in a direct or indirect manner (28). In other words; postbiotics refer to soluble by-products and metabolites secreted by the gut microbiota that exert biological activities on the host. This term is increasingly appearing in the scientific literature and commercial products (29). In 2021, the International Scientific Probiotic and Prebiotic Society defined postbiotics as "the preparation of non-living microorganisms and/or their components that provide health benefits to the host" (29). Postbiotic preparations can be easily and stably stored at room temperature for years without the need to take into account the progressive decrease in biological activity due to the loss of bacterial viability over time (29). These functional and physical properties of postbiotics have generated great interest among researchers

(30).

Probiotic-derived ferrichrome exerts a tumour-suppressive effect via the JNK signalling pathway (31). Ferrichrome was shown to be the molecule responsible for inhibiting the progression of colon cancer cells through JNK-DDTI3-mediated apoptosis and ferricrome may be a practical anti-tumor agent that can be used to inhibit the progression of colon cancer (31). Bioactive microbial compounds such as exopolysaccharide (EPS) preparations and cell-free supernatants (CFS) from Lactobacillus species have been suggested to be bioactive in some cancers. EPS reduced the proliferation of liver and GI tumor cell lines (32), while CFS preparations derived from Lactobacillus, Bifidobacterium and Faecalibacterium species induced cellular apoptosis, reduced tumor cell proliferation and activated anti-inflammatory signaling pathways in vitro models (33). An alternative postbiotic approach could use OMVs in a tumor modulating process. A modified OMV from *E. coli* has shown promising results as a cancer immunotherapeutic agent in colorectal cancer mouse models by accumulating in tumors and producing IFNg to enhance the antitumor response within the TME (34). An OMV-containing vaccine elicited a specific antitumor immune response with elimination of lung melanoma metastasis and inhibition of subcutaneous CRC growth (35). SCFA produced from probiotic fermentation is the best known example of postbiotics (36).

Examples of some of the positive effects of the products formed by the beneficial bacteria in the microbiota content on the prevention and treatment of colon cancer. In one study, the vitamin niacin (B3 vitamin) was shown to reduce DNA damage and carcinogenesis in various cancers, including breast, colon and oral cancers (37) and niacin deficiency was significantly more prevalent in Carcinoid tumor patients (38). Short-chain fatty acids (SCFA); acetate, propionate and butyrate) are the products of anaerobic bacterial fermentation of dietary fiber in the human colon. Among SCFAs, butyrate has multiple important roles as a key in colonic epithelial homeostasis: it is the main source of energy for colonocytes (39,40); may be protective against colon carcinogenesis (41); inhibits colon carcinogenesis (42,43); promotes the growth and proliferation of normal colonic epithelial cells (44,45); stimulates fluid and electrolyte absorption (46,47). According to one theory: SCFA and butyrate affect the cell cycle by inhibiting proliferation, inducing differentiation and cell death in human cancer cells (48-50). Based on this theory, a fermented nutrition approach has been proposed as an adjuvant in colon cancer treatment. There is evidence that propionate and butyrate exert an antiproliferative effect against colon cancer cells. Butyrate and propionate are also among the most potent living metabolites that induce cell differentiation and apoptosis. They are therefore protective against colorectal cancer (49,51,52). A study by Lương and Nguyễn showed a significant association between cancer and low levels of thiamine (B1 vitamin) in serum (52). A study examining the association between folate intake levels and the incidence of colorectal cancer suggests that higher folate intake levels lead to a reduction in one of the perceived risks associated with the development of colorectal cancer (53).

Fermented dairy products are gaining more attention due to their nutritional content and the lactic acid bacteria they contain, which improve the intestinal flora (54-56). Ayran, a special type of acidic milk drink, is popular in Turkey and many countries in Asia and the Middle East (57). In various countries of the world, buttermilk (ayran) -like products are produced by adding sugar

or fruit flavors and are called drinkable yoghurt, lactic drink or fermented milk drink (58). Using healthy human colon fibroblast (ATCC CRL-1459 Colon cell line) and human colorectal cancer (ATCC -CCL-224 colorectal adenocarcinoma cell line) cell lines; treated with normal buttermilk, buttermilk containing 1%, 2%, 3% and 4% postbiotic LTW 35 (*Streptococcus thermophilus ATA-LTC St140700, Bifidobacterium animalis ATA-BSLA0310, Lactobacillus acidophilus ATA-LAP1201* ferment extract lysate) respectively. After treatment, the effects of buttermilk components on cell lines will be tested by cell viability MTT assay.

MATERIAL AND METHODS

Healthy human colon fibroblast (ATCC CRL-1459 Colon cell line) and human colorectal cancer (ATCC -CCL-224 colorectal adenocarcinoma cell line) cell lines were used. CRL-1459 colon cancer cells were cultured in DMEM medium supplemented with 8% fetal bovine serum (FBS), penicillin - potassium (48 µg/ml), streptomycin sulfate (8,000 µg mL-1), amphotericin B (25 µg mL-1) and 1.2% L-glutamine. CRL-1459 colon fibroblast cells were cultured in DMEM medium supplemented with 8% FBS, penicillin-potassium (48 µg/ml), streptomycin (8,000 µg mL-1), amphotericin B (25 µg mL-1) and 1.2% L-glutamine. Cells were grown and stocked in an incubator at 37°C with 5% CO_2 and 90% humidity until cell density reached 80%. When cell density reached the desired level, CRL-1459 normal colon fibroblast cells were treated with various levels (0.005%-0.5%) of Tritonix 100 cytotoxicity. The cell group with a viability level of 56.1% at the end of the application was used in the study. Destroyed cells were treated with normal buttermilk and buttermilk containing 1, 2, 3 and 4% postbiotic LTW 35 (*Streptococcus thermophilus ATA-LTC St140700, Bifidobacterium animalis ATA-BSLA0310, Lactobacillus acidophilus ATA-LAP1201* ferment extract lysate) respectively. ATCC -CCL-224 colon cancer cells that reached sufficient majority were treated with normal buttermilk, buttermilk containing 1%, 2%, 3% and 4% postbiotic LTW 35 (*Streptococcus thermophilus ATA-LTC St140700, Bifidobacterium animalis ATA-BSLA0310, Lactobacillus acidophilus ATA-LAP1201* ferment extract lysate), respectively. After the treatment, cell viability was tested with MTT and CA 19-9 tumor marker was detected from cell lysates as a confirmation test.

RESULTS

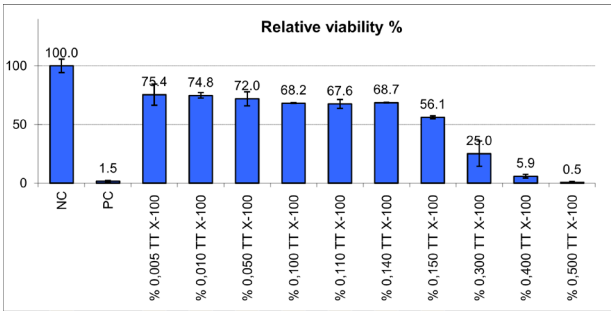

Figure 1. Viability graph of cells destroyed by cytotoxicity

Figure 1. shows the decrease in viability of CRL-1459 colon fibroblast cells after exposure to different concentrations (0.005%-0.5%) of TT X100. The graph indicates that as the concentration of TT X100 increases, the cell viability decreases significantly.

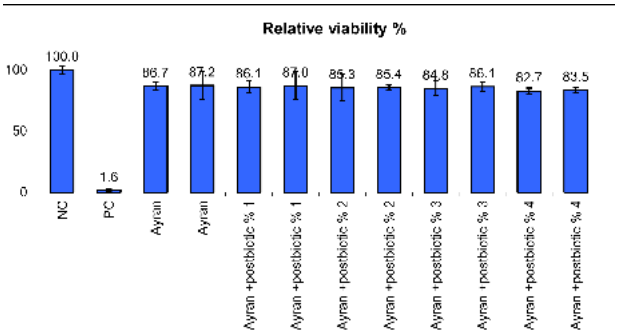

Figure 2. Viability graph after buttermilk treatment of cells destroyed by cytotoxicity

Figure 2 and figure (5-16) shows the viability rates of CRL-1459 colon fibroblast cells damaged by cytotoxicity after being treated with normal buttermilk and buttermilk containing postbiotic LTW 35. It is observed that the application of buttermilk and postbiotic content increased cell viability, and this effect is related to the concentrations used.

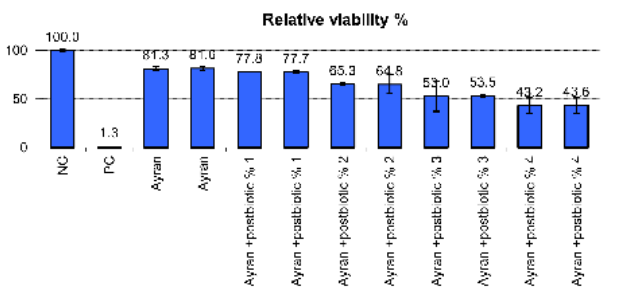

Figure 3. Viability graph after treatment of tumor cells

Figure 3 and figure (5-16) shows the viability rates of ATCC CCL-224 colorectal cancer cells after being treated with normal buttermilk and buttermilk containing 1%, 2%, 3%, and 4% postbiotic LTW 35. The applied buttermilk and postbiotic content reduced the viability of cancer cells, demonstrating potential anticancer effects.

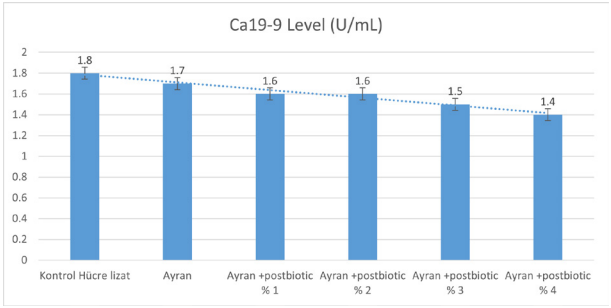

Figure 4. Ca 19-9 results graph

Figure 4. shows the changes in Ca 19-9 tumor marker levels in cell lysates obtained from ATCC CCL-224 colorectal cancer cells after treatment with normal buttermilk and buttermilk containing 1%, 2%, 3%, and 4% postbiotic LTW 35. The graph demonstrates a gradual decrease in Ca 19-9 levels as the postbiotic concentration increases, indicating the potential antitumor effect of the postbiotic.

Figure 5. Microscope view of destroyed cells

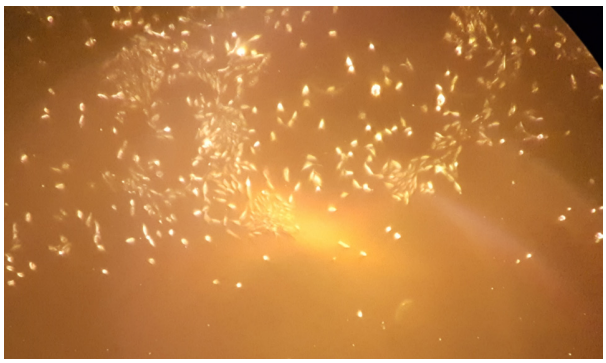

Figure 6. Microscope image of buttermilk-treated cells

Figure 7. Microscope image of buttermilk + 1% postbiotic-treated cells

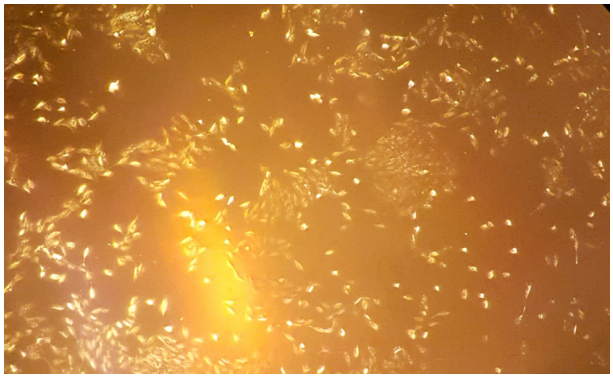

Figure 8. Microscope image of buttermilk + 2% postbiotic-treated cells

Figure 9. Microscope image of buttermilk + 3 % postbiotic-treated cells

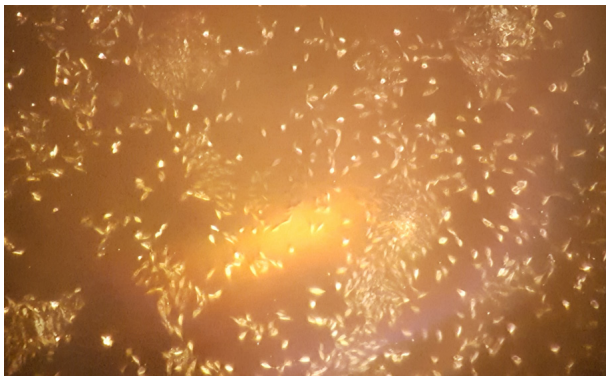

Figure 10. Microscope image of buttermilk + 4% postbiotic-treated cells

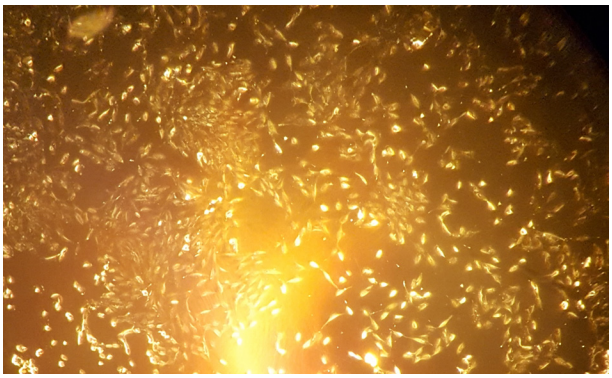

Figure 11. Microscope image of colon cancer cells

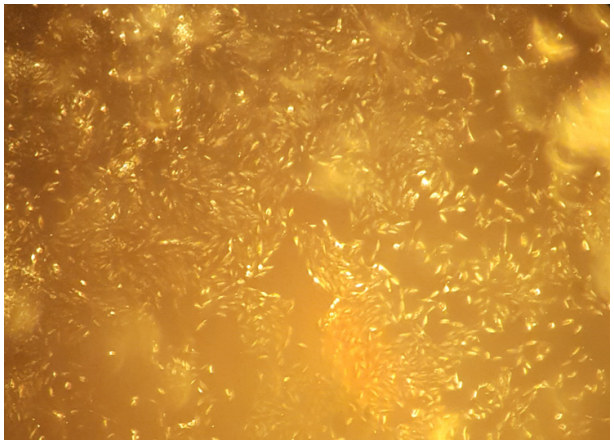

Figure 12. Microscope image of colon cancer cells after buttermilk supplementation

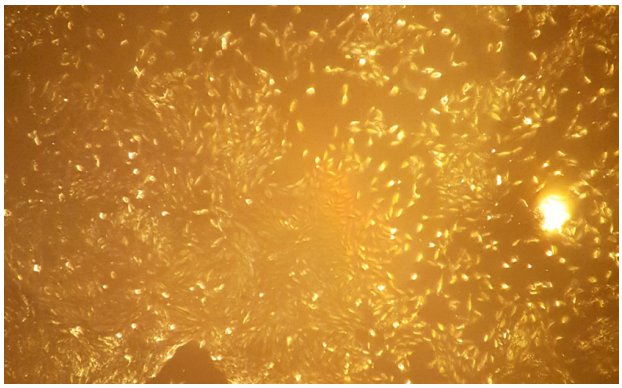

Figure 13. Microscope image of colon cancer cells after buttermilk and 1% postbiotic supplementation

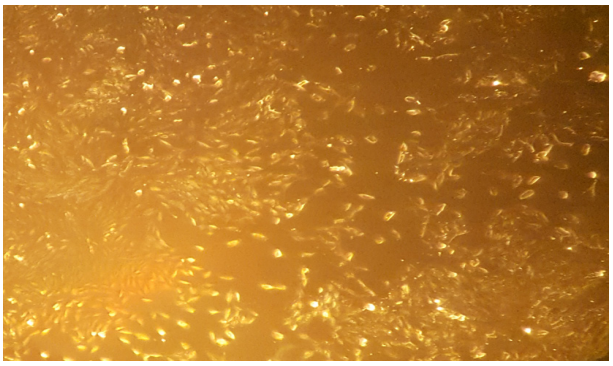

Figure 14. Microscope image of colon cancer cells after buttermilk and 2% postbiotic supplementation

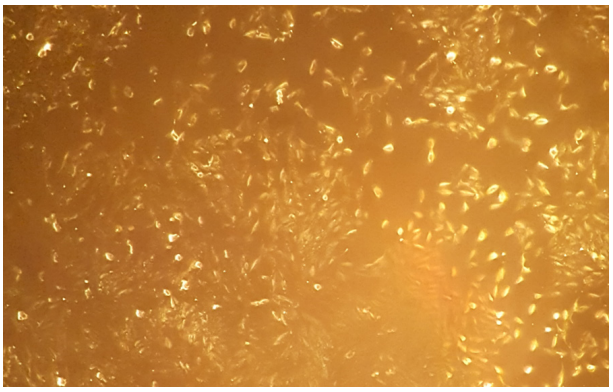

Figure 15. Microscope image of colon cancer cells after buttermilk and 3% postbiotic supplementation

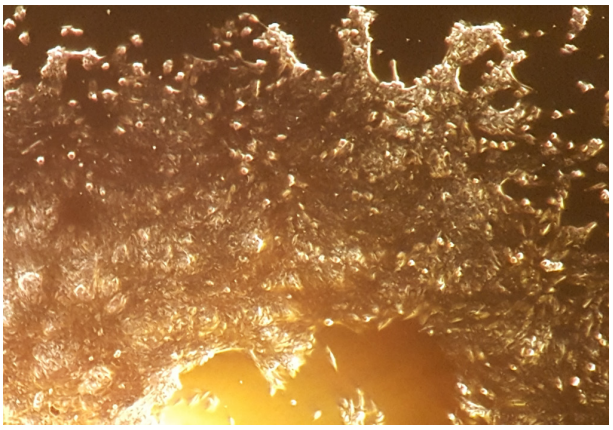

Figure 16. Microscope image of colon cancer cells after buttermilk and 4% postbiotic supplementation

DISCUSSION

The purpose of this study is to examine the effects of normal buttermilk and postbiotic LTW 35 (*Streptococcus thermophilus ATA-LTC St140700, Bifidobacterium animalis ATA-BSLA0310, Lactobacillus acidophilus ATA-LAP1201* ferment extract lysate) -enriched buttermilk on the viability of colon cancer cells. It also investigates changes in Ca 19-9 tumor marker levels to explore their potential cytoprotective and anticancer activities. Figure 1 highlights the dose-dependent decrease in the viability of CRL-1459 colon fibroblast cells after exposure to TTX 100. Figure 2 and figure (5-16) shows the improvement in viability of cytotoxicity-damaged fibroblast cells following treatment with buttermilk and postbiotic LTW 35, with effects linked to concentration levels. Figure 3 and figure (5-16) demonstrates that buttermilk and postbiotic LTW 35 reduce the viability of colorectal cancer cells, suggesting anticancer properties. Figure 4 and figure (5-16) illustrates a gradual decrease in Ca 19-9 tumor marker levels in cancer cells treated with buttermilk containing postbiotics, indicating the potential antitumor effects of the postbiotic. A study conducted by Bursalioglu (2021), which examined milk groups rich in probiotics, the effects of lyophilized human milk, mare milk, and cow colostrum on A549 lung cancer and MRC5 healthy lung cell lines were evaluated. The study found that human milk exhibited the strongest anticancer effects, followed by mare milk, while cow colostrum showed the least effect. Human and mare milk demonstrated antiproliferative effects on cancer cells without causing harm to healthy cells (27). Milk products contain a large number of probiotic bacteria and metabolites. The release of this content during the fermentation of probiotic bacteria in dairy products may prevent colorectal carcinogenesis (59). Moreover, van't Veer et al. hypothesized that high consumption

of fermented milk products (predominantly yogurt and buttermilk) may create protection against breast cancer (60). This study confirmed the effects of products containing the following ATA-coded postbiotics according to the literature. A study conducted by Aslan and colleagues demonstrated that postbiotics specifically inhibit odor-causing microorganisms while supporting and balancing the natural axillary microbiota. The findings revealed that formulations containing Lactobacillus ferment lysate extract were effective in reducing unpleasant odors by normalizing the microbiota (61). A study conducted by Gokce and Aslan investigated the antimicrobial potential of liposomal postbiotics in gel formulations. The optimized gel (LG1) showed effective antimicrobial activity against various pathogens, comparable to free postbiotics, while providing advantages such as controlled release, stability, and enhanced usability. These findings highlight the potential of liposomal postbiotics for pharmaceutical applications (62).

CONCLUSION

Increasing concentrations of TTX 100 significantly reduce the viability of CRL-1459 colon fibroblast cells, confirming a dose-dependent cytotoxic effect. Treatment with postbiotic LTW 35-enriched buttermilk improves the viability of cytotoxicity-damaged colon fibroblast cells, with higher concentrations providing greater restorative effects. Postbiotic LTW 35 reduces the viability of colorectal cancer cells in a concentration-dependent manner, demonstrating its potential anticancer properties. The observed decrease in Ca 19-9 tumor marker levels with postbiotic LTW 35 treatment highlights its potential role in reducing tumor activity in colorectal cancer cells.

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Domestic probiotics and postbiotic products with ATA code were placed on the shelves of BİM A.Ş, the food chain store brand of Turkey, and gained the favour of the consumer, and then the postbiotic ayran project, which is the subject of this research article, was born as a result of a joint exchange of ideas.

Conflict of interest statement: The authors report no conflict of interest.

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Ethical Decleration: No need.

Author Contribution: Concept: EB, LTÇ,BÇ, NG, HTK, İA, Design: EB, LTÇ,BÇ, NG, HTK, İA, Data Collection or Processing: G.K., Z.E., H.İ.K., H.E., Analysis or Interpretation: EB, LTÇ,BÇ, NG, HTK, İA, Literature Search: EB, LTÇ,BÇ, NG, HTK, İA, Writing: EB, LTÇ,BÇ, NG, HTK, İA

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