JJBS

Volume 18, Number 2,June 2025
ISSN 1995-6673
Pages 315 – 321
https://doi.org/10.54319/jjbs/180212

Jordan Journal of Biological Sciences

The Effect of *Memantine* and *Lactobacillus acidophilus* Supernatant on Apoptosis and Expression of Key Long non-coding RNAs in a Colon Cancer Cell Line

Paniz Erfanian Goli Zadeh Rauf Mohajer[1], Aras Rafiee[1,*], Soheila Zamanlui Benisi[2]

[1]*Department of Biology, CT.C., Islamic Azad University, Tehran, Iran;* [2] *Department of Biomedical Engineering, Central Tehran Branch, Islamic Azad University, Tehran, Iran*

Received: July 5, 2024; Revised: October 21, 2024; Accepted: November 26, 2024

Abstract

Alterations in the expression of long non-coding RNA (lncRNA) are linked to colon cancer progression. Recent studies suggest that *Memantine* (*ME*) and probiotics, such as *Lactobacillus acidophilus (LA)*, may have potential anti-cancer effects. This study investigates the impact of *Memantine*, *Lactobacillus acidophilus*, and their combination (*ME+LA*) on lncRNA expression, apoptosis, and the viability of HT-29 colon cancer cells. The MTT assay was used to evaluate cell viability and calculate IC50 using different concentrations of ME and *LA supernatant*. Expression levels of oncogenic lncRNAs (*SNHG16*, *NEAT1*, *CCAT2*, *MALAT1*, *H19*) were measured using Real-time PCR. Apoptosis and necrosis were analyzed via flow cytometry. The results demonstrated that higher concentrations of both *Memantine* and *LA supernatant* resulted in significantly reduced cell viability. Additionally, treatment with *ME* and *LA supernatant*, both individually and in combination, led to a reduction in the expression levels of oncogenic lncRNAs, induction of apoptosis and reducing cancer cell survival. The combination of *ME* and *LA* was found more effective. Therefore, combining *Memantine* and *LA supernatant* shows promise as a novel and effective treatment for colon cancer.

Keywords: Colon cancer, *Memantine*, *Lactobacillus acidophilus*, lncRNAs, Gene expression, Apoptosis

1. Introduction

Colon cancer is the third most prevalent cancer in the world to be diagnosed and presents a noteworthy health challenge, as indicated by the elevated incidence and mortality rates globally (Marcellinaro *et al.*, 2023). According to 2020 data, colon cancer affects 10% of the population and has a 9.4% death rate (Sung *et al.*, 2021). Despite multiple advancements in immunotherapy, chemotherapy, or radiotherapy, the adverse side effects of these therapies highlight the urgent need for innovative treatments. This necessity drives the exploration of novel and less invasive alternatives, such as probiotics and new drugs, which could potentially revolutionize colon cancer's preventive and therapeutic landscape.

Long non-coding RNAs (lncRNAs) have emerged as significant regulators in cancer biology, influencing gene expression and cellular behavior. Among these, several key lncRNAs, include Small Nucleolar R.N.A. Host Gene 16 (*SNHG16*), Nuclear Paraspeckle Assembly Transcript 1 (*NEAT1*), Colon Cancer-Associated Transcript 2 (*CCAT2*), Metastasis-Associated Lung Adenocarcinoma Transcript 1 (*MALAT1*), and H19. These lncRNAs are involved in various cellular processes, such as proliferation, apoptosis, and metastasis. For instance, *MALAT1*, known for its high conservation across mammals, regulates gene expression through its secondary structure and is associated with metastasis in multiple cancer types. Similarly, *NEAT1* plays a critical role in forming paraspeckles and is involved in the DNA damage response and cancer progression (Statello *et al.*, 2021). *CCAT2*, located upstream of the *MYC* gene, promotes tumor growth by regulating *MYC* expression (Ulitsky, 2016). *H19* functions as an oncogene in several cancers, including colon cancer, and regulates gene expression and cell growth (Ghafouri-Fard *et al.*, 2020). *SNHG16* has also been linked to poor prognosis in various cancers, contributing to tumorigenesis through its interaction with other molecular pathways (Chen *et al.*, 2020).

Recent studies suggest that the expression of lncRNAs can be influenced by dietary factors and the type of bacterial colonization in the colon. A study on patients with rectal cancer found that consumption of probiotics such as *L. acidophilus* was associated with improved expression of candidate lncRNAs (Khodaii *et al.*, 2022). Another research involving specific strains of *Bifidobacterium* and *Lactobacillus* has shown that they can

* Corresponding author.; e-mail: aras_rafiee@yahoo.com.

** **List of abbreviations:** CRC: colorectal cancer; LA: *Lactobacillus acidophilus*; Non-coding R.N.A.s (lncRNAs); *SNHG1:*Small Nucleolar R.N.A. Host Gene 16; *NEAT1:* Nuclear Paraspeckle Assembly Transcript 1; *CCAT2:* Colon Cancer-Associated Transcript 2; *MALAT1:* Metastasis-Associated Lung Adenocarcinoma Transcript 1

inhibit the growth of various types of cancer cells, including colorectal cancer cells (Śliżewska *et al.*, 2020). Probiotic metabolites, such as short-chain fatty acids (*SCFAs*), contribute to maintaining intestinal health, promoting apoptosis of cancer cells, and modulating immune responses (Kahouli *et al.*, 2013).

Similar to probiotics, *Memantine*, an *NMDA* receptor antagonist primarily used to treat Alzheimer's disease, has shown promising potential in cancer therapy (Maraka *et al.*, 2019). There are two types of glutamate receptors (GluRs): Metabotropic GluRs (mGluRs) and ionotropic GluRs (iGluRs). iGluRs form cation channels, allow Ca^{+2} and Na^{+} influx, and activate downstream apoptotic signaling pathways. One of the three iGluRs subcategories, N-methyl-Daspartate receptors (NMDARs), is particularly relevant to this research.

It has been demonstrated that NMDARs are more active in cancer cells than normal. NMDA receptors can activate cell growth cascades, such as the MAP kinase and ERK pathways, leading to the proliferation of non-neuronal cells including lung, colon, breast, bone, testis and pancreas (Yoon *et al.*, 2017). Therefore, NMDAR antagonists such as Memantine may possess anticancer properties (Gallo *et al.*, 2023; Shafiei-Irannejad *et al.*, 2021). Recent research indicates that *Memantine* can inhibit colorectal cancer, stop cancer cells from proliferating and cause them to undergo apoptosis (Mahboubi *et al.*, 2022). In a separate study, it was observed that the administration of Memantine increases the expression of Golgi glycoprotein 1 (GLG1). GLG1, in turn, reducing the bioavailability of fibroblast growth factor (FGF), a potent regulators of cell proliferation and differentiation during oncogenesis, by sequestering it intracellularly and extracellularly. Therefore, Memantine was found as a suppressor of malignant glioma and breast cancer cell growth by modulating the expression of GLG1, which functions as a traffic controller for FGF (Yamaguchi *et al.*, 2022). By disrupting cellular pathways that promote cancer cell survival, *Memantine* enhances the effectiveness of conventional therapies and offers a new avenue for targeted cancer treatment.

Considering the importance of developing effective treatments for colorectal cancer and the potential benefits of *Lactobacillus acidophilus (LA) supernatant* and *Memantine*, this study investigates their individual effects. Specifically, we assess the impact of *Memantine* and *LA supernatant* on apoptosis and the expression of five key lncRNAs (*SNHG16*, *NEAT1*, *CCAT2*, *MALAT1*, and *H19*) in colon cancer HT-29 cells. By examining these treatments, the study aims to provide insights into new therapeutic strategies for colorectal cancer.

2. Materials and Methods

2.1. Preparation of Memantine and LA supernatant

Memantine-coated tablets (5 mg) were purchased from Kimidaru Pharmaceutical Company (Iran). Two tablets (each, 5 mg) were crushed with a mortar, dissolved in 1 ml of DMEM solution, sterilized using a 0.2 nm filter, and stored in vials at 4℃ until use. Serial dilutions were then prepared to achieve various concentrations.

Lactobacillus acidophilus (ATCC 314) was procured from the Pasteur Institute. Bacterial cells were cultured on MRS agar. Then the colonies were inoculated into RPMI medium. The absorbance of the medium was monitored intermittently at 600 nm until reaching the optical density of 0.7 (equivalent to 0.5 MacFarland). The bacteria were then incubated for another 24 h at 37℃. After incubation, the culture was centrifuged at 3000 rpm for 15 minutes to isolate the supernatant. The supernatant metabolite was withdrawn with a syringe, passed through the 0.2 nm filter, freeze dried and finally stored in the refrigerator. At the time of use, 1 mg lyophilized powder was dissolved in 1 mL sterile DMEM.

HT-29 cells were treated with the IC50 values of both *LA* supernatant and memantine in Real-Time PCR and apoptosis assays.

2.2. Cell Line Preparation

The *HT-29* colon cancer cell line (IBRC C10071) was sourced from the National Center for Genetic and Biological Resources of Iran. These cells were maintained in DMEM culture medium.

HT-29 cells were maintained in *DMEM* supplemented with 20% *FBS* (Gibco, U.S.A.), 100 mg/ml streptomycin, 100 U/ml penicillin, and 2 mM L-glutamine at 37°C in a 5% CO2 incubator. The culture medium was refreshed every 24 to 48 hours. For cell passage, the medium was aspirated, and cells were detached by treating with 0.25% trypsin for 3-5 minutes. The trypsinization (Gibco, U.S.A.) process was halted by adding a medium containing serum. The cell suspension was then centrifuged at 1500 rpm for 5 minutes. The resulting pellet was resuspended in a fresh medium and transferred to new culture flask.

2.3. MTT Assay

HT-29 cells were seeded in 96-well plates and treated with different concentrations of *Memantine* (12.5, 25, 50, 100, 200 µg/ml) and *L. acidophilus (LA)* supernatant (250, 500, 1000, 2000, 4000 µg/ml). After 24 hours of incubation, MTT solution (5 mg/ml) was added to the cells, followed by an additional 3-hour incubation period. The medium was then discarded, and the resulting formazan crystals were dissolved in *DMSO*. Absorbance was measured at 570 nm using an *ELISA* reader. Cytotoxicity and cell viability were calculated using standard formulas. The IC50 values for *Memantine* and *L.A* supernatant were determined using the Pharm-PCS statistical package by analyzing the percentage of cytotoxicity.

2.4. Gene Expression Analysis by Real-Time PCR

We evaluated the impact of *L. acidophilus* supernatant on gene expression by real-time PCR. Briefly, 4×10^5 *HT-29* cells were seeded in 6-well plates containing 2ml of complete cell culture medium overnight. Then cells were treated with IC50 concentration of both memantine and *LA* supernatant (separately) for 24 hours. Negative controls received an equal volume of DMEM medium.

We followed the real-time procedures on the basis of MIQE guidelines (Bustin *et al.*, 2009). RNA extract from *HT-29* cells was performed using the RNX-Plus reagent (CinnaGen) following standard protocols. OD at A_{260}/A_{280} (purity) was investigated by a spectrophotometer. The RNA (OD ~ 1.9-2) was then used to synthesize cDNA with the miScript II RT kit without interruption (CinnaGen) (Table-1). 500 ng/20 µl RNA concentration was used for cDNA synthesis. cDNA was stored at 20℃.

The expression of target lncRNAs (*SNHG16*, *NEAT1*, *MALAT1*, *H19*, *CCAT2*) was quantified using SYBR Green-based Real-Time PCR with specific primers designed from the NCBI database and IDT online software. The mean Cq values of Gapdh and HPRT1 were used as the reference genes for normalization.

Table1. List of Genes and Primers:

Gene	Primer Sequence (5'-3')	Product Length (bp)	Melting Temperature (Tm)	Designed on exons
Gapdh	F: GTGGTCTCCTCTGACTTCAAC	96	57.97	7,8
	R: GGAAATGAGCTTGACAAAGTGG		58.09	
HPRT1	F: AAGGGTGTTTATTCCTCATGGAC	105	58.40	2,3
	R: AGCACACAGAGGGCTACAA		58.55	
SNHG16	F: CAGAATGCCATGGTTTCCCC	142	58.38	3,4
	R: TGGCAAGAGACTTCCTGAGG		59.12	
NEAT1	F: GTACGCGGGCAGACTAACAC	101	57.37	1-1
	R: TGCGTCTAGACACCACAACC		57.36	
MALAT1	F: AATGTTAAGAGAAGCCCAGGG	150	59.02	1 -1
	R: AAGGTCAAGAGAAGTGTCAGC		59.12	
H19	F: GGATCCAGTTAGAAAAAGCCCGGGCT	113	57.78	2,3
	R: ACGCGTGCTGTAACAGTGTTTATTGA		58.00	
CCAT2	F: CCACATCGCTCAGACACCAT	100	57.44	1-1
	R: ACCAGGCGCCCAATACG		57.58	

Primers were ordered from a commercial supplier (Sinaclon) and verified for specificity using NCBI BLAST and GeneRunner software.

2.5. Real-Time PCR Procedure:

We performed Real-time PCR reactions in a total volume of 25 µl. The reaction mixture included 12.5 µl of Master mix (Bioneer, Korea), 1 µl of forward primer (10 mM), 1 µl of reverse primer (10 mM), 1 µl of cDNA (0.1-1 µg), and 9.5 µl of DEPC-treated water. The reactions were performed using a Bioneer Exicycler 96. The thermal profile used for the real-time PCR reactions is detailed in Table 2. The qPCR data was analyzed by Livak method ($2^{-\Delta\Delta CT}$).

Table 2. Real-Time PCR Thermal Profile

Step	Function	Temperature (°C)	Time	Repeat
1	Initial Denature	95.0	15 minutes	1
2	Denature	95.0	20 seconds	40
3	Anneal	58.0	20 seconds	40
4	Extend	72.0	20 seconds	40
5	Melt Curve	55.0 to 94.0	1 second	1
6	End			

2.6. Apoptosis Analysis by Flow Cytometry

HT-29 cells were treated with the IC50 concentration of *Memantine* and *LA* supernatant to assess apoptosis. The cell staining was carried on with Annexin V-FITC and Propidium Iodide (PI) using the Annexin V-FITC kit (eBioscience, USA). The data were analyzed to determine the percentage of apoptotic cells, distinguishing between early apoptotic, late apoptotic, necrotic, and viable cells by Flow cytometry.

2.7. Statistical Analysis

Statistical analysis was conducted using REST 2009 and SPSS 16. Data analysis involved one-way ANOVA, with Tukey's HSD post-hoc test used to determine significant differences. Gene expression levels were evaluated using the CT method, and the results were visualized with GraphPad software. Data were presented as mean ± standard deviation (SD), with significance delimited at $P<0.05$.

3. Results

3.1. Cell Viability of the HT-29 Cell Line Under Various Treatments for 24 Hours

Figure 1 illustrates the results of the MTT assay conducted on HT-29 cells treated with *Memantine* and LA supernatant. Both *Memantine* and *Lactobacillus acidophilus (LA) supernatant* treatments significantly impacted the survival of cancer cells. *HT-29* cells were exposed to various concentrations of supernatant (250, 500, 1000, 2000, and 4000 µg/ml) and *Memantine* (12.5, 25, 50, 100, and 200 µg/ml). Cell viability was measured using the MTT assay over 24 hours. The results indicate that increasing concentrations of the *LA supernatant* led to a significant decrease in cell viability, with 44% of cancer cells eliminated at 4000 µg/ml after 24 hours ($P<0.001$) (Figure 1a). Concentrations of 1000 and 2000 µg/ml also significantly reduced cell viability ($P<0.05$, $P<0.01$), whereas 250 and 500 µg/ml did not have a significant effect. The IC50 value for *LA supernatant was* determined to be 5187.86 µg/ml, the concentration needed to achieve a 50% reduction in cell viability. Similarly, higher concentrations of *Memantine* significantly decreased cell viability, with 69% of cancer cells eliminated at 200 µg/ml after 24 hours ($P<0.001$) (Figure 1b). All concentrations of *Memantine* significantly reduced cell viability. The calculated IC50 value for *Memantine* was 35.21 µg/ml.

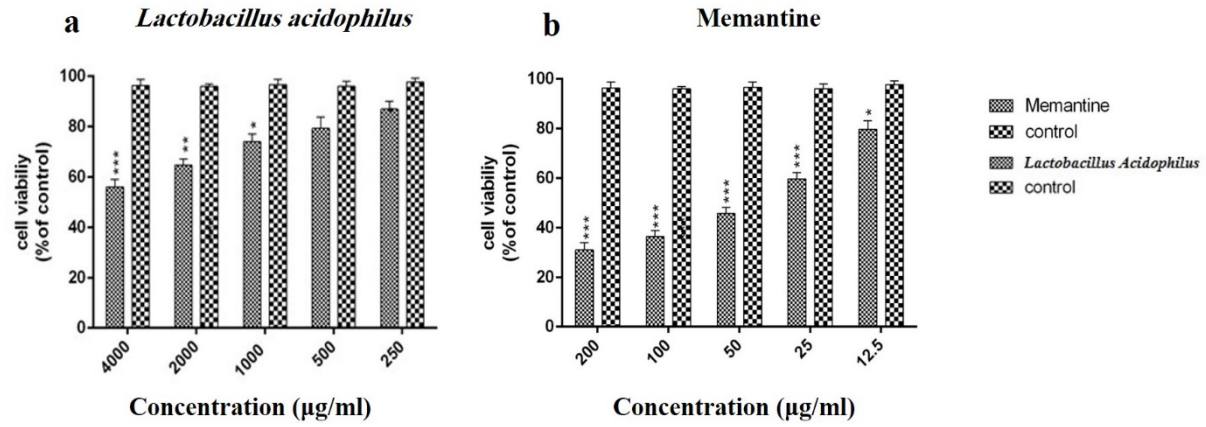

Figure 1. (a) Bar chart showing the cell viability of *HT-29* cells treated with *Lactobacillus acidophilus* supernatant. (b) Bar chart showing the cell viability of HT-29 cells treated with *Memantine*.

3.2. Expression of lncRNAs SNHG16, NEAT1, MALAT1, H19, and CCAT2 by Real-Time PCR

Prior to PCR, cDNA concentrations were measured and normalized using a NanoDrop spectrophotometer, with the reference genes. Figure 2 presents the comparative and average gene expression results for *HT-29* cells treated with *Memantine*, *Lactobacillus acidophilus*, and their combination. The relative gene expression of *SNHG16*, *NEAT1*, *MALAT1*, *H19*, and *CCAT2* in *HT-29* cells is illustrated under different treatments compared to the control. A significant reduction in gene expression was observed across all treatments compared to the control, with the most pronounced decrease noted in the combination treatment of *Memantine* and *LA supernatant.* Overall, these results indicate that treatment with *Memantine* and *LA supernatant*, both individually and particularly in combination, leads to a reduction of the oncogenic genes *SNHG16*, *NEAT1*, *MALAT1*, *H19*, and *CCAT2* in *HT-29* cancer cells.

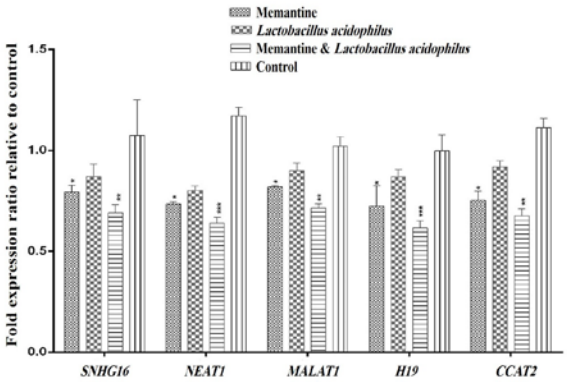

Figure 2. Relative fold expression levels of *SNHG16*, *NEAT1*, *MALAT1*, *H19*, and *CCAT2* genes in *HT-29* cells treated with IC50 concentrations of *Memantine*, *LA supernatant*, and their combination compared to control. Data are presented as mean ± SD based on three replicates. (* indicates P<0.05, ** indicates P<0.01, *** indicates P<0.001).

3.3. Flow Cytometry Analysis Using Annexin-V Kit

Figure 3 presents the flow cytometry results for apoptosis induction in control cells (panel b) and *HT-29* cells treated with the IC50 concentration of the *LA supernatant (*panel a). The results indicate that 98.4% of the control cells are viable, which is expected as they were not treated with the *LA* or *Memantine*. The percentage of early and late apoptosis was 1.41% and 0.13%, respectively, and a minimal 0.22% of the cells underwent necrosis due to the collection and preparation process, which is negligible compared to the healthy and viable cells.

The flow cytometry results demonstrated the effects of IC50 concentrations of *LA supernatant*, *Memantine* and their combination on *HT-29* cells. We found that combination treatment induced the highest levels of late apoptosis (28.20%), significantly higher than either treatment alone (*Memantine* at 11.32% and *LA supernatant at* 9.76%). Early apoptosis was also significantly elevated in cells treated with *Memantine* (15.60%) and the combination (5.14%) compared to the control (1.52%). While the necrosis rates remained relatively low and statistically insignificant across all groups, the combination treatment yet showed a slight increase (3.10%). We suggest a synergistic effect between *LA supernatant* and *Memantine*, enhancing apoptotic cell death while maintaining low necrosis levels, highlighting the potential for combined therapeutic strategies in targeting cancer cells.

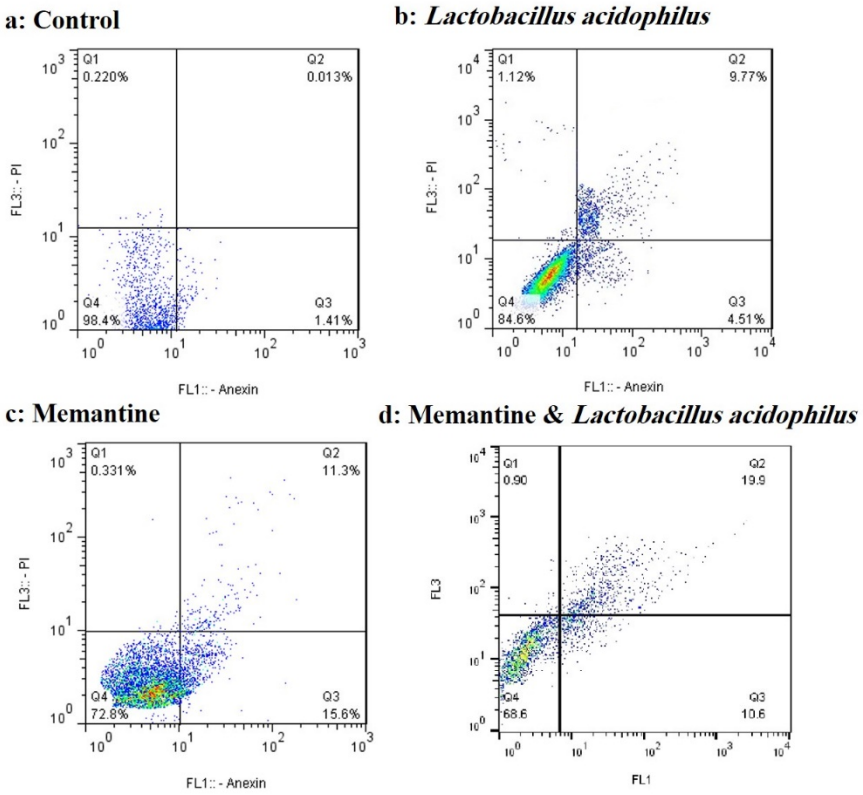

Figure 3. Flow cytometry analysis of apoptosis induction in *HT-29* cells. (a) Control cells without any treatment. (b) *HT-29* cells were treated with an IC50 concentration of the probiotic *Lactobacillus acidophilus (LA) aupernatant*. (c) *HT-29* cells were treated with an IC50 concentration of *Memantine*. (d) *HT-29* cells were treated with *Memantine* and *LA supernatant*.

4. Discussion

Colorectal cancer ranks among the most prevalent types of cancer, with its incidence rapidly increasing among both men and women in recent years. Each year, over 1 to 2 million new cases are diagnosed, and the disease is responsible for over 600,000 deaths. The prevalence is particularly higher among men and older adults (Rawla *et al.*, 2019). Early screening poses challenges, as over 80% of colorectal cancers derive from adenomatous polyps, but it can identify patients two to three years before symptoms appear.

Memantine, an *NMDA* receptor antagonist, has demonstrated potential anti-cancer effects by increasing mitochondrial complex 1 activity while reducing complex 5 activity. A study indicated that *Memantine* disrupts *glutamate* synthesis in prostate cancer cells, leading to cell death. *Memantine*'s effects on *NMDA* receptors, which play critical roles in various physiological processes due to their high calcium flow and magnesium block, suggest its potential in cancer therapy (Gulsah Albayrak *et al.*, 2021). *Lactobacilli* are catalase-negative, Gram-positive rods that ferment carbohydrates to produce lactic acid and exhibit immunomodulatory properties that benefit for maintaining microbial balance. However, some strains can transfer antibiotic-resistance genes, necessitating careful evaluation of their probiotic properties. Their benefits include antimicrobial activity, antibiotic resistance, acid and bile tolerance, and stability (Di Cerbo *et al.*, 2016).

This study assessed the cytotoxic effects of *Lactobacillus acidophilus supernatant* and *Memantine* using the MTT assay, revealing dose-dependent reductions in cancer cell viability. *Memantine* exhibited significantly greater cytotoxicity than the *LA supernatant*, with a 150-fold difference in IC50 values. Hence, memantine exhibits high cytotoxicity against cancer cells and can impede key metabolic pathways like glutamate synthesis in cancer cells while sparing normal cells. If its low toxicity effect on normal cells is confirmed, memantine could potentially be considered as a future option for cancer treatment, along with probiotics.

Previous studies have also indicated *Memantine*'s dose-dependent cytotoxic effects on various cancer cells (G Albayrak *et al.*, 2018).

We demonstrated that *Memantine* and *Lactobacillus acidophilus* significantly induced apoptosis in *HT-29* cells, with the combination treatment showing the highest levels of late apoptosis. Gene expression analysis indicated significant downregulation of oncogenic lncRNAs *SNHG16*, *NEAT1*, *MALAT1*, *H19*, and *CCAT2* following treatments, with the combination treatment yielding the most substantial reductions. These findings align with other studies that have reported the apoptotic and antiproliferative effects of *LA* and *Memantine* (Isazadeh *et al.*, 2020; Robinson *et al.*, 2020).

The observed effects may be due to *Memantine's* antagonistic effect on NMDA receptors, which disrupts intracellular calcium signaling and activates apoptotic pathways, particularly the mitochondrial (intrinsic) pathway. This calcium imbalance triggers *cytochrome c* release from mitochondria, subsequently activating *caspases* responsible for apoptosis (Calvo-Rodriguez *et al.*, 2020). On the other hand, *Lactobacillus acidophilus* has been found to modulate the gut microbiota and produce *SCFAs* metabolites with anti-inflammatory and

anti-cancer properties (Thananimit *et al.*, 2022). These metabolites can inhibit histone deacetylases (*HDACs*), resulting in histone hyperacetylation and increased tumor suppressor gene expression (Li *et al.*, 2022). Additionally, probiotics can bolster the host's immune response by promoting the production of anti-inflammatory cytokines, which may contribute to their anti-cancer properties (Wu *et al*., 2021).

The combination treatment appears to exert a synergistic effect, potentially through the simultaneous modulation of multiple pathways. The downregulation of oncogenic lncRNAs *SNHG16*, *NEAT1*, *MALAT1*, *H19*, and *CCAT2* suggests these treatments may interfere with the regulatory networks controlling gene expression and cellular proliferation. For instance, lncRNAs are famed for interaction with chromatin-modifying complexes and transcription factors, and their altered expression can lead to significant changes in the transcriptional landscape of cancer cells (Emam *et al.*, 2022; Segal and Dostie, 2023).

Furthermore, the apoptotic properties of the *LA supernatant* and *Memantine* were investigated using annexin flow cytometry. The results showed that both the *LA supernatant* and *Memantine* induced apoptosis, increasing early and late apoptosis in the treated cells. While there was no significant difference in the necrosis rates between cells treated with *LA supernatant* or *Memantine*, the apoptotic effects were noticeable. A study demonstrated that supernatants from *Lactobacillus acidophilus* and *casei* strains reduced cancer cell proliferation and increased apoptosis. Additionally, their supernatants could induce necrosis and reduce cell migration and invasion, which aligns with our apoptotic findings (Mirzadeh *et al.*, 2024). Another study examined the protective effects of oral *Lactobacillus casei BL23* in mice with induced CRC. The findings showed significant protection against CRC, reduced histological scores, and modulated immune response, as indicated by increased cytokine *IL-22* and caspase expression. This suggests the potential of *L. casei BL23* in developing probiotic-based strategies against CRC (Jacouton *et al.*, 2017).

In conclusion, we highlight the potential of *Memantine* and *Lactobacillus acidophilus supernatant* as effective agents for inducing apoptosis and reducing the viability of HT-29 colorectal cancer cells. The combined treatment significantly downregulated oncogenic lncRNAs, indicating a synergistic effect. These findings suggest that *Memantine* and *Lactobacillus acidophilus* could be promising candidates for colorectal cancer therapy. Take note of the following suggestions for future studies: 1- Explore the cytotoxic effects of *Lactobacillus acidophilus* and *Memantine* on other cancer cell lines and compare their effects on normal cell viability, 2- explore the impact on the expression of other lncRNAs involved in cancer, 3- assess the effects of different chemical drugs and probiotic supernatants on cancer cell lines, 4- investigate the role of different tumor microbiome in regulating cancer and develop microbiome-targeted therapies, and eventually 5- conduct further in vivo and clinical trial studies.

5. Conflict of Interest Statement

The authors declare no conflict of interests. The research was conducted independently without external funding or influence.

Acknowledgments

The authors would like to express their sincere gratitude to Pasteur Institute of Iran (IPI), Stem cell and Cell therapy research center for providing the necessary facilities and resources to conduct this research; special thanks go to Dr. Zahra Shahi, Dr. Farhad Riazi-Rad, Dr.Asal Katebi and Dr. for their invaluable guidance and support throughout the study. Additionally, appreciation is extended to all the staff members of the Microbiology Laboratory for their assistance and collaboration.

References

Albayrak G, Konac E, Dikmen AandBilen C. 2018. Memantine induces apoptosis and inhibits cell cycle progression in LNCaP prostate cancer cells. *Hum. Exp. Toxicol*., **37 (9)**: 953-958.

Albayrak G, Korkmaz FD, Bali EBandBagriacik EU. 2021. Antitumorigenic Effect of Memantine via Interfering Glutamate Metabolism in Mouse 4T1 Breast Tumor Model. *Anti-Cancer Agents Med. Chem*., **21 (12)**: 1575-1580.

Bustin SA, Benes V, Garson JA, Hellemans J, Huggett J, Kubista M, et al. 2009. T**he MIQE Guidelines: Minimum Information for Publication of Quantitative Real-Time PCR Experiments,** Oxford University Press.

Calvo-Rodriguez M, Kharitonova EKandBacskai BJ. 2020. Therapeutic strategies to target calcium dysregulation in Alzheimer's disease. *Cells*., **9 (11)**: 2513.

Chen L, Qiu C-H, Chen Y, Wang Y, Zhao J-JandZhang M. 2020. LncRNA SNHG16 drives proliferation, migration, and invasion of lung cancer cell through modulation of miR-520/VEGF axis. *Eur Rev Med Pharmacol Sci*., **24 (18)**: 9522-9531.

Di Cerbo A, Palmieri B, Aponte M, Morales-Medina JC, Iannitti T. 2016. Mechanisms and therapeutic effectiveness of lactobacilli. *J. Clin. Pathol*., **69 (3)**: 187-203.

Emam O, Wasfey EFandHamdy NM. 2022 .Notch-associated lncRNAs profiling circuiting epigenetic modification in colorectal cancer. *Cancer Cell Int.*, **22 (1)**: 316.

Gallo S, Vitacolonna AandCrepaldi T. 2023. NMDA receptor and its emerging role in cancer. *Int. J. Mol. Sc*., **24 (3)**: 2540.

Ghafouri-Fard S, Esmaeili MandTaheri M. 2020. H19 lncRNA: roles in tumorigenesis. *Biomed.*, **123**: 109774.

Isazadeh A, Hajazimian S, Shadman B, Safaei S, Bedoustani AB, Chavoshi R, et al. 2020. Anti-cancer Effects of Probiotic Lactobacillus acidophilus for Colorectal Cancer Cell Line Caco-2 through Apoptosis Induction. *Pharm. Res.*, **27 (2)**: 262-267.

Jacouton E, Chain F, Sokol H, Langella PandBermudez-Humaran LG. 2017. Probiotic strain Lactobacillus casei BL23 prevents colitis-associated colorectal cancer. *Front. immunol.*, **8**: 1553.

Kahouli I, Tomaro-Duchesneau CandPrakash S. 2013. Probiotics in colorectal cancer (CRC) with emphasis on mechanisms of action and current perspectives. *J.Med. Microbiol.*, **62 (8)**: 1107-1123.

Khodaii Z, Mehrabani Natanzi M, Khalighfard S, Ghandian Zanjan M, Gharghi M, Khori V, et al. 2022. Novel targets in rectal cancer by considering lncRNA–miRNA–mRNA network in response to Lactobacillus acidophilus consumption: a randomized clinical trial. *Sci. Rep.*, **12 (1)**: 9168.

Li P, Chen G, Zhang J, Pei C, Chen Y, Gong J, et al. 2022. Live *Lactobacillus acidophilus* alleviates ulcerative colitis via the SCFAs/mitophagy/NLRP3 inflammasome axis. *Food Funct.*, **13 (5)**: 2985-2997.

Mahboubi N, Shafiei-Irannejad V, Kahyaei_aghdam MandSoraya H. 2022. Memantine enhances the cisplatin-induced apoptosis in A2780 ovarian cancer cells via CyclinD1 and hTERT inhibition. *Process Biochem.*, **122**: 69-75.

Maraka S, Groves MD, Mammoser AG, Melguizo-Gavilanes I, Conrad CA, Tremont-Lukats IW, et al. 2019. Phase 1 lead-in to a phase 2 factorial study of temozolomide plus memantine, mefloquine, and metformin as postradiation adjuvant therapy for newly diagnosed glioblastoma. *Cancer Cell Int.*, **125 (3)**: 424-443.

Marcellinaro R, Spoletini D, Grieco M, Avella P, Cappuccio M, Troiano R, et al. 2023. Colorectal cancer: current updates and future perspectives. *J. Clin. Med.*, **13 (1)**: 40.

Mirzadeh MA, Eslami M, Ghanbari A, Zarbakhsh S, Yosefi SandPakdel A. 2024. Coadministration of doxorubicin with vitamin D3, *Lactobacillus acidophilu*s, and *Lactobacillus casei* in the 4T1 mouse model of breast cancer: anticancer and enteroprotective effects. *Med Oncol*., **41 (5)**: 111.

Rawla P, Sunkara TandBarsouk A. 2019. Epidemiology of colorectal cancer: incidence, mortality, survival, and risk factors. *Prz. Gastroenterol*., **14 (2)**: 89.

Robinson EK, Covarrubias SandCarpenter S. 2020. The how and why of lncRNA function: An innate immune perspective. *Biochim Biophys Acta Gene Regul Mech*., **1863 (4)**: 194419.

Segal DandDostie J. 2023. The talented LncRNAs: meshing into transcriptional regulatory networks in cancer. *Cancers (Basel)*., **15 (13)**: 3433.

Shafiei-Irannejad V, Abbaszadeh S, Janssen PMandSoraya H. 2021. Memantine and its benefits for cancer, cardiovascular and neurological disorders. *Eur. J. Pharmacol.*, **910**: 174455.

Śliżewska K, Markowiak-Kopeć PandŚliżewska W. 2020. The role of probiotics in cancer prevention. *Cancers.*, **13 (1)**: 20.

Statello L, Guo C-J, Chen L-LandHuarte M. 2021. Gene regulation by long non-coding RNAs and its biological functions. *Nat. Rev. Mol. Cell Biol.*, **22 (2)**: 96-118.

Sung H, Ferlay J, Siegel RL, Laversanne M, Soerjomataram I, Jemal A, et al. 2021. Global cancer statistics 2020: GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 countries. *CA Cancer J Clin*., **71 (3)**: 209-249.

Thananimit S, Pahumunto NandTeanpaisan R. 2022. Characterization of short chain fatty acids produced by selected potential probiotic Lactobacillus strains. *J Biomolecules*., **12 (12)**: 1829.

Ulitsky I. 2016. Evolution to the rescue: using comparative genomics to understand long non-coding RNAs. *Nat. Rev. Genet.*, **17 (10)**: 601–614.

Wu Z, Pan D, Jiang M, Sang LandChang B. 2021. Selenium-enriched lactobacillus acidophilus ameliorates dextran sulfate sodium-induced chronic colitis in mice by regulating inflammatory cytokines and intestinal microbiota. *Front. Med.*, **8**：.716816

Yamaguchi F, Hayakawa S, Kawashima S, Asakura TandOishi Y. 2022. Antitumor effect of memantine is related to the formation of the splicing isoform of GLG1, a decoy FGF- binding protein. *Int. J. Cancer*., **61 (1)**: 1-11.

Yoon W-S, Yeom M-Y, Kang E-S, Chung Y-A, Chung D-SandJeun S-S. 2017. Memantine induces NMDAR1-mediated autophagic cell death in malignant glioma cells. *J Korean Neurosurg Soc.*, **60 (2)**: 130-137.