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Investigating the Synergism of *Lactobacillus Plantarum* Cell Lysate Supernatant and Carboplatin on the Induction of Cell Death and Expression of *Bax* and *Bcl-2* Genes in the SK-OV-3 Cell Line

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Abstract

Introduction: This study aimed to investigate the synergistic and separate cytotoxicity effects of carboplatin (a chemotherapy medication) and *Lactobacillus plantarum* cell lysate supernatant (CLS) in the SK-OV-3 ovarian cancer cell line and the expression change of apoptotic *Bax* and anti-apoptotic *Bcl-2* genes.

Materials and Methods: *L. Plantarum* CLS at concentrations of 0.05, 0.1, 0.15, 0.25, 0.5, 1, 1.25, and 1.5 mg/ml and carboplatin at concentrations of 0.1, 0.5, 1, 2, 2.5, 5, 10, 20, 40, and 50 µg/ml were prepared. Separate and synergistic toxicity effects of carboplatin and *L. Plantarum* CLS at different concentrations were investigated on the SK-OV-3 cell line at intervals of 24, 48, and 72 h using the MTT method. Also, the expression level of *Bax* and *Bcl-2* genes in the treated SK-OV-3 cell line was analyzed using Real-time PCR.

Results: In the current study, the highest increases of toxicities in the separate and synergistic application of carboplatin and *L. Plantarum* CLS were seen after 48-h treatment against cancer cells. These toxicities were time-dependent, and with increasing time from 24 to 72 h, their cytotoxicity increased. The results of the MTT test showed that drug and probiotics synergism could cause the highest decrease in the survival rate of the SK-OV-3 cell line in 72, 48, and 24 h, respectively. This synergism led to a 2.8-fold

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decrease in the expression of the *Bcl-2* gene and a 3.5-fold increase in the *Bax* gene compared to the control group. Carboplatin alone decreased *Bcl-2* by 1.4 times and increased *Bax* by 1.5 times, and CLS alone decreased *Bcl-2* by 3.2 times and increased *Bax* by 1.2 times.

Discussion and Conclusion: We concluded the combination of an *L. Plantarum* CLS (0.1 mg/ml) and carboplatin (0.5 µg/ml) has induced apoptosis in the SK-OV-3 cell line. *L. plantarum* .CLS can be used as adjunctive therapy with carboplatin through increased toxicity and changes in apoptosis gene expressions.

Key words: Ovarian cancer, *L. Plantarum* CLS, Carboplatin, SK-OV-3, *Bcl-2*, *Bax*

Introduction

Generally, cancer is a disorder in which abnormal cells are reinforced by escaping the conventional regulations of cellular division. Despite the normal cells, we witness cancer cells experience abnormal cell division and growth; consequently, it brings many difficulties for tissues and organs (1). The exact cause of this phenomenon is unclear, and it has been proposed genetics and other factors that disrupt the activity of cells cause disorders in the cell nucleus. Ovarian cancer is one of the most important causes of cancer death in women in developed countries (2). The most common malignant ovarian cancers in postmenopausal women are of the epithelial type (3). SK-OV-3 is a human ovarian cancer cell line with epithelial-like morphology and, like other cancer cell lines, is often sensitive to tumor necrosis factor (TNF) and cytotoxic drugs (4). Drug delivery as a therapeutic tool has grabbed the attention of researchers within the past decades due to drug bioavailability, control of administrated toxicity doses for obtaining the optimal response, and effortless transportation of the chemotherapy drugs into the target cells of the organ (5). A considerable number of studies have focused on innovative approaches for delivering the drugs to the cancer cell, like using nanoparticles conjugated with anticancer compounds and synergism of different chemotherapy medications with apoptosis-inducer substances and microorganisms (6). Platinum-based drugs, such as carboplatin, cisplatin, and oxaliplatin have the property of disrupting the genome and are used before and after surgery to prevent disease recurrence and improve the overall survival rate (7). These drugs prevent the growth and development of cancer by disrupting the growth of cancer cells and are usually used together with other anti-cancer drugs to treat ovarian cancer, and other types of malignancy, such as lung, testicle, head, neck, and brain tumors (8, 9). Clinical trials in advanced ovarian cancer have shown that carboplatin is similar in activity to cisplatin and causes significantly less cytotoxicity, neurotoxicity, and nephrotoxicity (10).

Apoptosis is programmed cell death, which is involved in a variety of biological events and is the last way to escape from the cells becoming cancerous (11). Cell apoptosis leads to the fragmentation of cell cytoplasm, and nucleus, which are swallowed by phagocytes (12). Many genes are involved in causing or not causing apoptosis through changes in their expression level in the development of cancer cells. The *Bcl-2* family is the most recognized protein family engaged in the regulation of apoptotic cell death, consisting

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of pro-apoptotic and anti-apoptotic members (13, 14). *Bcl-2* is an anti-apoptotic gene encoding the Bcl-2 protein that prevents apoptosis either by preventing the release of mitochondrial apoptogenic factors like apoptosis-inducing factor (AIF) and cytochrome c into the cytoplasm or by sequestering proforms of death-driving cysteine proteases called caspases (15). On the opposite, other members of the *Bcl-2* family like *Bak* and *Bax*, trigger the release of caspases from death antagonists and induce the release of mitochondrial apoptogenic factors into the cytoplasm (16). Therefore, this family acts as a critical life-death decision point within the common pathway of apoptosis.

Probiotics help stimulate the growth of beneficial intestinal bacteria or reduce the pathogenicity of harmful microbes (17). They also produce antimicrobial compounds such as nisin, which prevent the formation of infection by pathogens (18). Probiotics, mainly lactic acid bacteria (LAB), are advantageous microorganisms that bring many benefits to humans and animals. Improving the immunity system, food digestion in the gastrointestinal tract, absorption of minerals, and wound healing as well as decreasing blood pressure cholesterol, and triglyceride are some of the properties of these valuable microbes (19). Previous studies have reported that some probiotics have anti-cancer activities by neutralizing the toxicity of substances (e.g., ammonia and secondary bile acids) that cause genetic damage in the intestine (20-23). Also, probiotics inhibit the initial phase of carcinogenesis by reducing epithelial pressure on active carcinogens. Diets based on probiotics may be used as an adjuvant anti-cancer therapy during chemotherapy (24). These groundbreaking results suggest that the use of probiotics is a promising strategy for maximizing the efficacy of cancer immunotherapy (25). This study aimed to study the cytotoxic synergistic and separate effects of carboplatin drug (a chemotherapy medication) and probiotics (*Lactobacillus plantarum*) cell lysate supernatant (CLS) in different concentrations on the ovarian cancer cell line, SK-OV-3. Moreover, their impacts on the expression of *Bcl-2* and *Bax* genes were investigated.

Materials and Methods

Preparation and Cultivation of SK-OV-3 Ovarian Cancer Cells: SK-OV-3 ovarian cancer cell line (NCBI Code: C209) was purchased from the cell bank of Pasteur Institute in Tehran, Iran, and the passage was performed as follows. The medium in the flask was discarded under the laminar hood (Jaltajhiz Co, Iran) with the help of a pipette, and the cells were washed with 2 ml of phosphate-buffered saline (PBS). Then, 2 ml of trypsin / EDTA 1x (Biosero Co, Iran) was added to each 25x2 cm flask and kept in an incubator (Memmert Co, Germany) at 37°C, 5% CO_2, and 96% humidity for 3-5 min to increase the trypsin performance. Afterward, flasks were observed under the inverted microscope (Olympus Co, japan), as detached cells were spherical after trypsinization and separation from the bottom of the flask. About 0.5 ml of Dulbecco's Modified Eagle's Medium (DMEM) (Bioidea Co, Iran) with 10% fetal bovine serum (FBS) was added to each flask to prevent the trypsin effect. The obtained cell suspension was transferred into a 15 ml

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Falcon to be centrifuged at 12000 rpm at 25°C for 2 min. In the next step, the supernatant was removed, and 1 ml of DMEM medium containing penicillin and streptomycin antibiotics and 10% FBS was added to make a cell suspension. Then, 4 ml of DMEM medium was mixed with suspension previously transferred to the flask and was incubated (previous conditions). The cell medium was changed every 48 h due to the presence of acidic metabolites and achieving a high cell density (about 70-80%) during the growth phase. After the passaging step, the cells were pipetted and placed a drop of the suspension on the Neubauer slide. With the help of a microscope, the number of cells was counted according to the suspension volume. Then, the falcon containing the cell suspension was centrifuged (Sunny Co, Japan) at 1500 rpm for 7 min, and then the cell sedimentation was kept at -20 °C.

Preparation, Cultivation, and *L. Plantarum* CLS: *L. plantarum* bacterium (purchased from the National Center of Genetic and Biological Resources of Iran) was cultured anaerobically (by GasPak jar) in a de Man, Rogosa, and Sharpe (MRS) broth (Merck, USA) at 37 °C. The cultured bacteria in the logarithmic phase were centrifuged for 2 min at 9000 rpm, the supernatant was discarded, and 1 gr (wet weight) of bacterial cells was floated in 5 ml of PBS buffer. Then, the cells were lysed with the help of an ultrasonic probe sonicator (Hiescher Co, Germany). After filtration of the lysed bacteria by a 0.22 µm syringe filter, in order to ensure the high efficiency of the bacterial lysis process, the optical density (OD) of the samples was read with a spectrophotometer (Bosch and Lomb Co, USA) at a wavelength of 600 nm.

SK-OV-3 Ovarian Cancer Cells Treatment and MTT Assay: After the cultivation of the SK-OV-3 cell line in the flask, it was essential to separate the cells from the flask for cell suspension preparation. Therefore, trypan blue staining was used to determine the viable percentage of SK-OV-3 cell suspension. For this purpose, 20 µl of trypan blue solution was added to 20 µl of SK-OV-3 cell suspension. About 10 µl of the obtained solution was placed on a slide, and counting was done under a microscope (10 X magnification). The living cells do not allow the dye to enter the cell due to their intact membrane and selective permeability, while the dye enters the dead cells and turns them blue. MTT assay is a colorimetric method to measure the proliferation and viability of cells. In this method, the activity of the mitochondrial dehydrogenase enzyme is checked. The enzyme activity in living cells leads to the production of a yellow water-soluble tetrazolium crystal, to purple formazan, insoluble in water. To obtain the best concentration for treatment by the three compounds studied in this research, including carboplatin (Sobhan Co. Iran), *L. plantarum* CLS, and their synergism, $5x10^3$ SK-OV-3 cells were cultured in each well of a 96-well plate. Different concentrations of the CLS and carboplatin (Table 1), were added to the seeded cells separately, synergistically, and an MTT assay test based on the protocol (Bioidea Co, Iran) was performed as follows: after 24 h. 10 λ of MTT assay solution was added to each well, and then the plate was incubated at 37 °C for 4 h. After incubation, the medium of the wells was discarded, 100 λ of DMSO was added to the sediment, and the plates were gently shaken for 10 min. Finally, the OD of each well was measured using an ELISA reader (Biotech Co, USA) at a wavelength of 570.

RNA Extraction and Preparation of

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cDNA: For the preparation of RNA from SK-OV-3 ovarian cancer cells, the protocol based on a total RNA extraction kit (Parstous Co, Iran) was carried out. The vials containing RNAs were stored at – 80 °C. The quality and homogenization of the RNA samples were evaluated by the NanoDrop One C device (Thermo Scientific Co, US). A cDNA synthesis kit was used (Parstous Co, Iran) to obtain cDNA from the extracted RNAs according to the following protocol. For this purpose, a total volume of 20 µl was supplied, including; 2 µl enzyme mix; 10 µl buffer; and 8 µl RNA template, and the mixture was performed by a vortex (Fisher Scientific Co, US) at 25 °C for 5 min. A total volume of 20 µl was added to each microtube, including 2 µl oligo dT, 2 µl dNTP (10mM), 1 µl RT enzyme, 1.5 µl $MgCl_2$, 4 µl 5X PrimeScript buffer, 9 µl distilled water and 0.5 µl RNasin (40 Unit/µl). PCR reaction tubes were inserted at 25 °C for 10 min in a thermocycler (Jenabio Co, Germany) for primers binding. Then, samples remained in a thermocycler at 47 °C for 60 min to synthesize cDNA, and finally for 5 min at 85 °C to stop RT enzyme activity. The final product was kept at -20 °C for the next steps. The sequence of *Bax* and *Bcl-2* genes as target and GAPDH gene as reference was obtained from the National Center for Biotechnology Information (NCBI) website, and then specific primers with accuracy and specificity were designed by the Gene Runner Software v3.0.1. Finally, the designed primers were synthesized (Sinaclon Company, Iran). The reverse and forward primer sequences used in this study are summarized in the following Table 2.

Expression changes of *Bax* and *Bcl-2* genes using Real-Time PCR: In this research, the SYBER green method (For binding to DNA), primers of *Bax* and *Bcl-2* genes, as well as cDNA were obtained from SK-OV-3 ovarian cancer cells applied in two separate reactions and duplicated. Despite being priceless, sensitive, and effortless use of SYBER green, due to binding to two strands such as primer dimer and other non-specific bands, results are estimated to be higher than the original concentration. Thereby, melting curve analysis is used to minimize the presence of non-specific products. To investigate the decrease or increase in the expression of the studied genes, we compared expression change with the reference control genes (GAPDH) was used as a reference gene. Generally, four cDNAs were analyzed: cDNAs related to treatments of 1) carboplatin, 2) lysed bacteria, 3) synergism of lysed bacteria and carboplatin, and 4) control cDNA. The PCR reaction was performed in a Real-Time PCR (Qiagen Co, US) in a total volume of 20µl in a microtube for each gene, including 1 µl cDNA, 1 µl forward primer, 1 µl reverse primer, 10 µl 2X SYBR green master mix (Parstous Co, Iran), and 7 µl DEPC water. Cycles conditions of the reaction were as follows; 1) One cycle at 95 °C for 3 min as the first step for denaturation, 2) 40 cycles at 95 °C for 20 sec, 40 cycles at 53 °C for 20 sec, 40 cycles at 72 °C for 10 sec, and 3) 72 °C for 3 min for elongation.

Statistical analysis of data: The statistical analysis of this study was done using SPSS version 25 software, and the results were analyzed with a one-way analysis of variance (ANOVA). The difference in the target gene expression between the control and treated samples was calculated using Rotor-Gene and SPSS software, the method of Tukey's HSD post-hoc test ($P<0.05$). Real-time PCR data analysis was carried out based on threshold cycle comparison. The difference in the threshold cycles was obtained through cells treated with agents (lysed bacteria and carboplatin) and untreated cells. Using the specific formula ($\Delta\Delta Ct$), the ratio of the target gene to the reference gene of GAPDH was calculated

through $2^{-\Delta\Delta Ct.}$ After performing the reaction and obtaining Ct using Rest 2009 software, we calculated ΔΔCt of each sample.

ΔCt= Ct target-Ct reference

ΔΔCt= ΔCt test sample - ΔCt control sample

Relative expression (fold Change) = $2^{-\Delta\Delta Ct}$

The expression of *Bax* and *Bcl-2* genes in the SK-OV-3 cell line was calculated through the following equation:

$$\text{Fold change} = \frac{(\text{ct Treat} - \text{ct Control}) \text{ of target genes } (Bax \text{ or } Bcl2)}{(\text{ct Treat} - \text{ct Control}) \text{ of reference gene } (GAPDH)}$$

Results

Assessment of the Cytotoxicity of the Carboplatin and *L. plantarum* CLS: Toxicities of carboplatin and *L. plantarum* CLS synergistically and separately were investigated after 24, 48, and 72h of incubation. The graph of cell viabilities regarding treatments is shown in Figure 1. The results showed half inhibitory concentration (IC_{50}) for the separate carboplatin were at a concentration of 2.5, 2, and 1µg/ml, when they were 0/5, 0.25, and 0.15 mg/ml for *L. Plantarum CLS* against cancer cells, respectively after 24, 48, and 72 h. The synergistic effect of carboplatin and *L. plantarum* CLS revealed IC_{50} at concentrations of 2 µg/ml of carboplatin + 0.25 mg/ml of *L. plantarum* CLS (after 24h), 0.5 µg/ml of carboplatin + 0.1 mg/ml of *L. plantarum* CLS (after 48h), and 0.5 µg/ml of carboplatin + 0.1 mg/ml of *L. plantarum* CLS (after 72h). Based on IC_{50} results, the highest toxicities of separate carboplatin and *L. plantarum* CLS were seen at 72-h treatment, while synergism had the highest toxicity at 48-h treatment, and then this amount did not experience change within 72 h. Obtained IC50s of separate and synergism of carboplatin and *L. plantarum* CLS are summarized in Table 3.

Table 1- Different Concentrations of Carboplatin and *L. plantarum* CLS

Substance										
Carboplatin (µg/ml)	50	40	20	10	5	2.5	2	1	0.5	0.1
L. plantarum CLS (mg/ml)			1.5	1.25	1	0.5	0.25	0.15	0.1	0.05
Synergism of carboplatin (µg/ml) and *L. plantarum* CLS (mg/ml)			30 Cpb + 1. 5 Mg/ml Bac	20 Cpb + 1. 25 Mg/ml Bac	10 Cpb + 1 Mg/ml Bac	5 Cpb + 0. 5 Mg/ml Bac	0. 25 Cpb + 0. 25 Mg/ml Bac	0. 15 Cpb + 0. 15 Mg/ml Bac	0. 5 Cpb + 0/1 Mg/ml Bac	0. 1 Cpb+ 0. 05 Mg/ml Bac

Legend: Cpb (carboplatin), Bac (*L. plantarum* CLS).

Table 2- The Sequence of Primers Used in Real-time PCR

Primers	Sequence	Tm
Bax Forward	5′ TCCCCCCGAGAGGTCTTTT3′	54 °C
Bax Reverse	5′ CGGCCCCAGTTGAAGTTG3′	54 °C
Bcl-2 Forward	5′ TTGGCCCCCGTTGCTT3′	55 °C
Bcl-2 Reverse	5′ CGGTTATCGTACCCCGTTCTC3′	55 °C
GAPDH Forward	5′ GAAGGTGAAGGTCGGAGTC3′	54 °C
GAPDH Reverse	5′ GAAGATGGTGATGGGATTTC3′	54 °C

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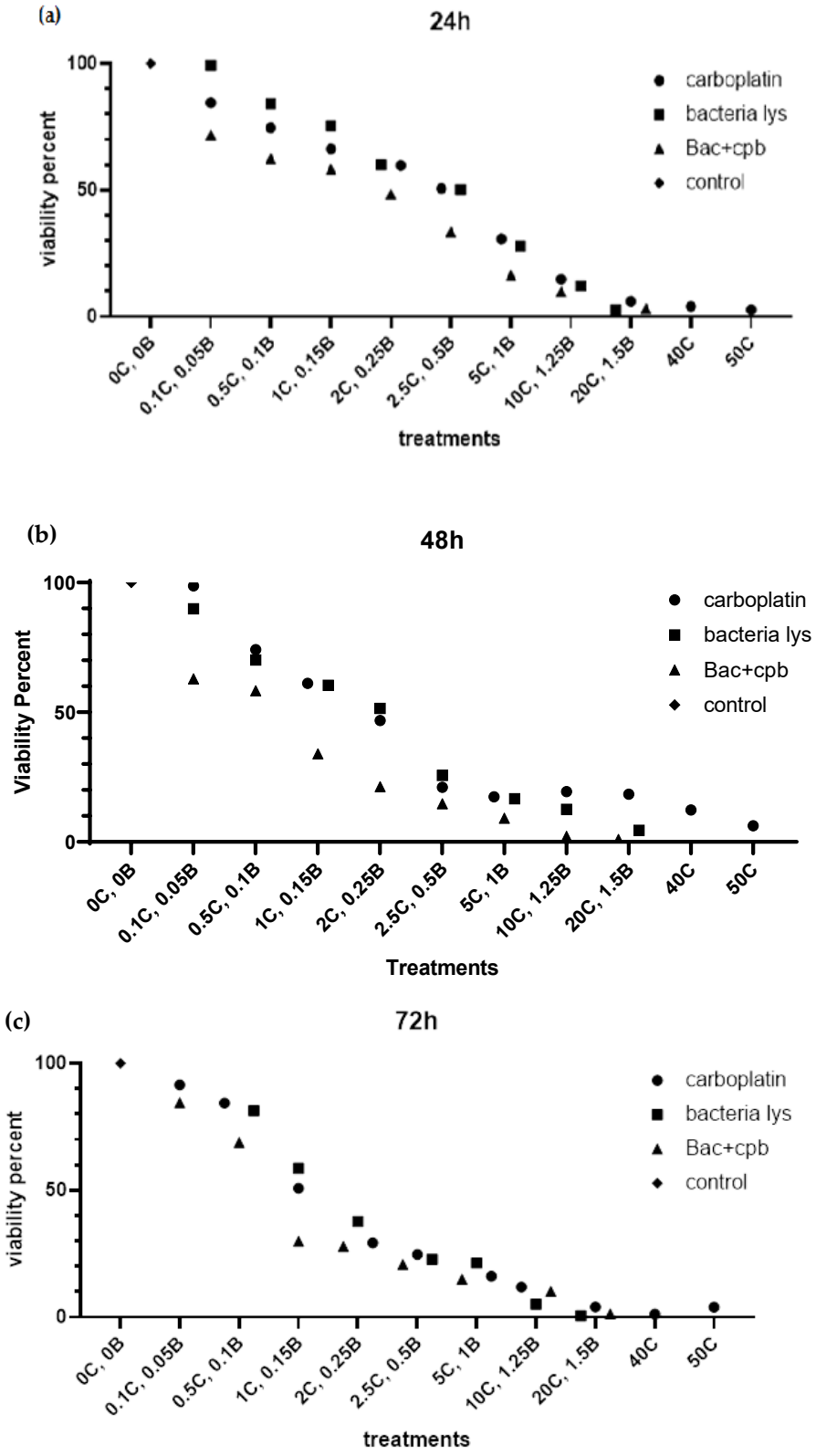

Fig. 1- SK-OV-3 cell viability percentage after (a) 24-hour, (b) 48-hour, and (c) 72-hour treatment with carboplatin and *L. plantarum* CLS in different treatment groups
Legend: cbp (carboplatin), Bac (*L. plantarum* CLS)

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Table 3- Obtained IC_{50}s of Separate and Synergism of Carboplatin and *L. plantarum* CLS against SK-OV-3

	Cpb			*L. plantarum* CLS			Cpb+ *L. plantarum* CLS		
	24h	48h	72h	24h	48h	72h	24h	48h	72h
IC_{50}	2. 5 ug/ml	2 ug/ml	1 ug/ml	0. 5 mg/ml	0. 25 mg/ml	0. 15 mg/ml	2ug/ml +0. 25mg/ml	0. 5ug/ml +0. 1mg/ml	0. 5ug/ml +0. 1mg/ml

The treatments were carried out at a concentration of 0.5 ug/ml of carboplatin, and 0.1 mg/ml of *L. plantarum* CLS. The data analysis was performed using the threshold cycle and investigating the synergism of carboplatin, and *L. plantarum* CLS on the induction of cell death. Figure 2 shows the graphs of the melting curve markers of the investigated genes in the polymerase chain reaction. Since fluorescent dyes such as cyber green were used in Real-time PCR, we could examine the melting curve for each gene. The figures demonstrate that each gene has its melting curve, so the curves of a gene in all samples should match and all curves should be single-peaked. Therefore, the graphs show that the amplification of the above genes was specific, and the absence of a non-specific product confirms the correctness of the reaction. According to the results, synergism of carboplatin *L. plantarum* CLS had the most tangible effect on *Bcl-2* anti-apoptosis gene expression and led to a decrease in its expression by about 2.8-fold change compared to the control. It is concluded that the synergistic effect of two substances compared to separate treatments has led to a decrease in *Bcl-2* gene expression and as a result increased apoptosis. Analysis of the data was done using SPSS version 25 software for a significant difference. Based on the ANOVA test results, the Sig value was greater than 0.05, therefore there is no significant difference among the *Bcl-2* gene expression in the control and treatment groups separately and synergistically (Figure 3 a). Also, about the *Bax* gene (coding an apoptosis protein), we witnessed an increase of 3.5-fold change in its expression under synergism of carboplatin and *L. plantarum* CLS compared to the control (Figure 3 b). Separate use of lysed bacteria and carboplatin also increased the *Bax* gene expression by 1.2 and 1.5 fold changes compared to the control. A significant difference was not obtained for the *Bax* gene among control and treatment groups considering ANOVA test results.

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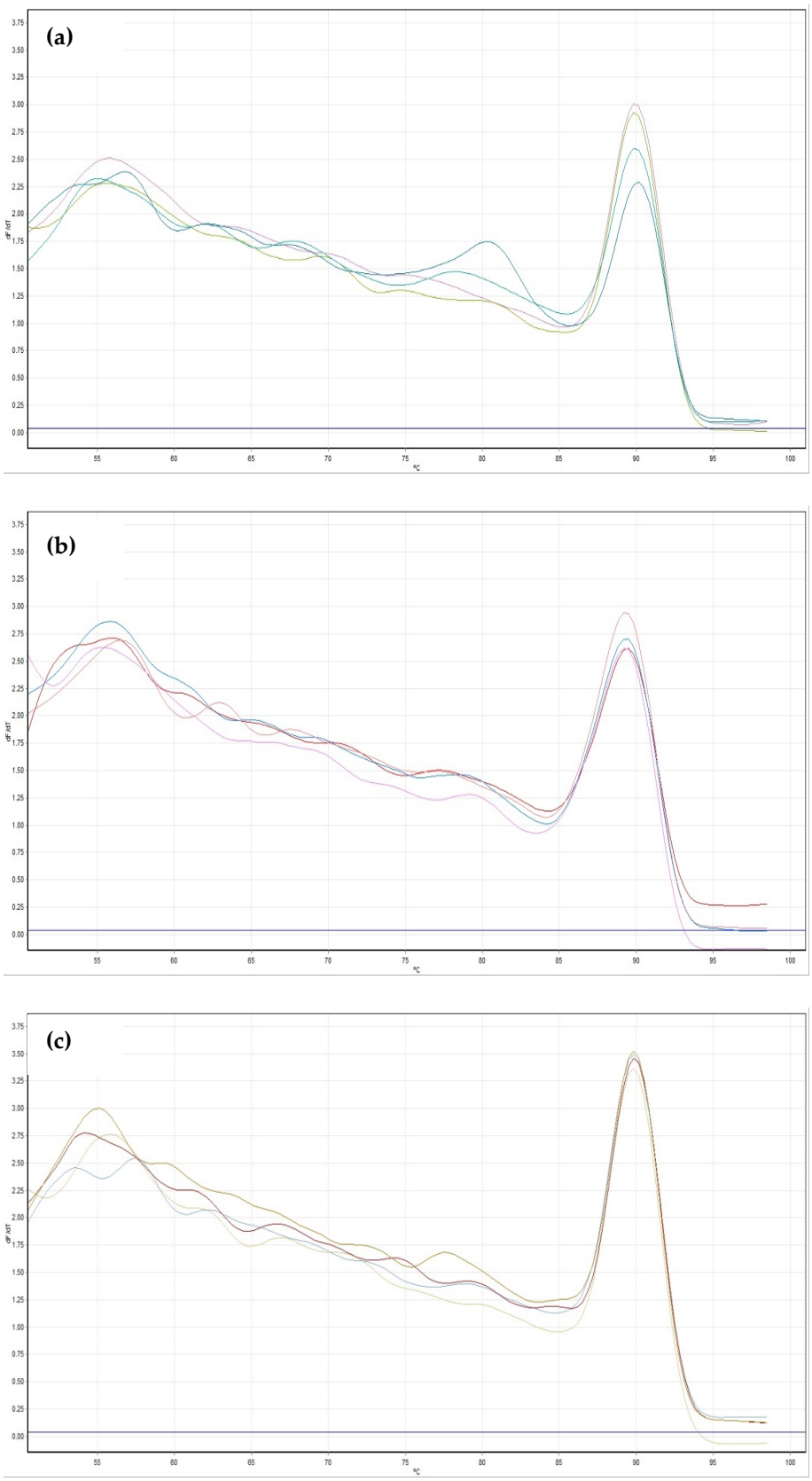

Fig. 2- The Real-time PCR melting curves of (a) *GAPDH*, (b) *bcl-2*, and (c) *Bax* genes

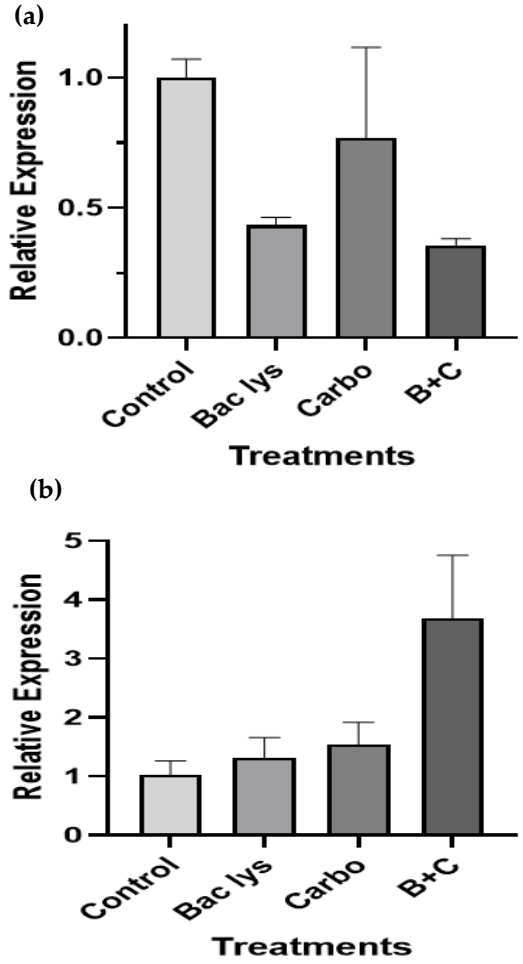

Fig. 3- Gene expression changes of (a) *Bcl-2* and (b) *Bax* in the concentration of 0.1 mg/ml *L. plantarum* CLS and 0.5 µg/ml of carboplatin in four different treatment conditions
Legend: Carbo (Carboplatin), Bac lys (*L. plantarum* CLS), and B+C (Carboplatin + *L. plantarum* CLS)

Discussion

Cytotoxicity testing, a biological evaluation, and screening test use tissue cells *in vitro* to observe cell growth, reproduction, and morphological effects (26). The effectiveness of anticancer drugs is measured by their ability to recognize cancer cells and selectively induce their apoptosis (27). Thus, it is vital to find compounds with anti-cancer properties in chemotherapy drugs that are involved in apoptosis. In the current study, carboplatin and *L. plantarum* CLS experienced a decrease in IC_{50} by 1.5 ug/ml and 0.35 mg/ml, respectively, between 24 and 48 h. This decrease occurred in the synergistic effect between 12 and 24 h by 1.5 µg/ml of carboplatin and 0.15 mg/ml of *L. plantarum* CLS, as we did not observe any changes in 72-h treatment. It seems that synergistic combinations of two or more agents can overcome toxicity and other side effects associated with high doses of single drugs, by either countering biological compensation, sparing doses on each compound, or accessing context-specific multi-target mechanisms. In the present study, these toxicities increases can be attributed to the chemical or physical agents interacting with living organisms that may trigger perturbations in cell function and/or structure or that may initiate repair mechanisms at the molecular, cellular, and/or tissue levels (28). Also, it seems that this increase may depend on the route of exposure, and environmental conditions like temperature and pH (29). Recent publications suggest main mechanisms behind the protection of probiotics against chemotherapy toxicity are the inactivation of cytokines (30) and oncogenes by the inhibition of nuclear translocation of β-catenin and nuclear factor kappa B ($NF\text{-}_kB$) (30), suppressor gene activation, and the cell cycle maintenance (31). The impacts of probiotics on various cancers rely on different various mechanisms, such as anti-mutagenic properties, modification of differentiation process in tumor cells, production of short-chain fatty acids, alteration of tumor gene expressions, and anti-carcinogenic effects. Also, reduction of intestinal pH to reduce microbial activity, activation of the host's immune system, inhibition of the bacteria that convert pro-carcinogens to carcinogens, and alteration of colonic motility and transit time can be considered as other mechanisms, as Javanmard argued (32). *In vitro* studies demonstrated that probiotic intervention induces cancer cells apoptosis and inhibits

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their proliferation. For example, in human models, it has been reported that *L casei shirota* decreased breast cancer incidence, and the use of fermented milk products was inversely associated with breast cancer incidence (32).

Previous studies have not worked on the synergistic effect of carboplatin and probiotics CLS in toxicity against cancer cells, and the current study is the first investigation. However, the synergism of similar drugs and probiotic CLS against various cancer cells has been studied. Sharaf et al. (31) investigated the combination administration of celecoxib and *Lactobacillus rhamnosus* GG, *Lactobacillus acidophilus* in 1,2-dimethylhydrazine-induced colon carcinogenesis. They reported ten days of consumption of this synergism up-regulated pro-apoptotic *Bax* and tumor suppressor p53 and effectively decreased tumor multiplicity, tumor burden, down-regulated proto-oncogene K-ras, and the expression of anti-apoptotic *Bcl-2*, compared to the control. Our results confirm theirs when the synergism of carboplatin and *L. plantarum* has led to a 2.8-fold decrease in the expression of the *Bcl-2* gene and a 3.5-fold increase in the *Bax* gene. Sharaf and Shukla (30) assessed the conjunction celecoxib and *L. rhamnosus* GG, *Lactobacillus. acidophilus* in DMH -induced colon carcinogenesis and reported reduced aberrant crypt foci (ACF) count and the expression of pro-carcinogenic molecular markers. Also, previous studies have reported that probiotics CLS in combination with other chemotherapy drugs can prevent chemotherapy-related diarrhea (33) and decrease the doses of drugs (34). In a similar study, Wu et al. (35) reported probiotic mixture protects against cisplatin (a chemotherapy drug)-induced intestine injury, exhibiting both anti-inflammatory and antiemetic properties. These results were closely related to the re-establishment of intestinal microbiota ecology and normalization of the dysbiosis-driven 5-hydroxytryptamine (5-HT, serotonin) overproduction.

The anticancer property and toxicity of the separate probiotic strains widely have been studied. Nisin, produced by *Lactococcus* and *Streptococcus* species during fermentation, and as an antimicrobial agent, has many applications in the food industry. This bacterial metabolite also reveals anticancer abilities against different types of cancer. For example, Sadri et al. (36) worked on the cytotoxicity of nisin against human umbilical vein endothelial cells (HUVECs), human cervical cancer cell lines (HeLa), and human ovarian carcinoma cell lines (OVCAR-3 and SK-OV-3) using an MTT assay. They argued the treatment of cervical cancer cells with 12 µM nisin significantly increased the *Bax* and/or *Bcl-2* genes expression (4.9 fold) and decreased reactive oxygen species (ROS) levels by 1.7 fold. When concentration-dependent cytotoxicity of nisin had low toxicity against normal endothelial cells, IC_{50} values of 11.5–23 µM were obtained against cancer cell lines. Our study is in agreement with Sadri's research when toxicities of *L. plantarum* extracts against SK-OV-3 cell lines were observed separately and synergistically with carboplatin. Gene expression of *Bax* and *Bcl-2* experienced an increase and decrease, respectively, while Sadri reported increases for both genes. Previously, Norouzi et al. (37) achieved the same results regarding nisin cytotoxicity against LS180, SW48, and HT29 cell lines in colorectal cancer through down-regulating *CEA*, *CEAM6*, *MMP2F*, and *MMP9F* genes. Tukenmez et al. also showed that *Lactobacillus delbrueckii* subsp. *bulgaricus* B3-EPS inhibited proliferation and induced apoptosis by increasing the expression of *Bax*, caspase 3, 6, and 9 and decreasing the

expression of *Bcl-2* and Survivin protein (38). Cell-bound exopolysaccharides (cb-EPS) derived from *L. acidophilus* 606 inhibited the growth of HT-29 colon cancer cells by activating the *Bax* gene (38). The results of these studies were in line with the results of our study on gene expression changes. In this study, probably the toxicity has a direct correlation with time, as the time exposure goes by, the IC_{50s} have decreased from 24 to 48 h. Furthermore, CLS and carboplatin are reactive to DNA and cellular proteins and the primary mode of action is mostly through cross-linking of DNA strands, inhibiting the replication of DNA and transcription of RNA.

Conclusion

The synergism of carboplatin drug and *L. plantarum* CLS had a toxicity activity against SK-OV-3 ovarian cancer cells as their viabilities were decreased. *L. plantarum* CLS induced apoptosis through different mechanisms. Also, the separation and synergism of substances did not make a significant difference in the expression of the pro-apoptotic gene *Bax* and the anti-apoptotic gene *Bcl-2*; however, the expression of the *Bax* gene increased significantly. It seems that simultaneous treatment methods such as taking medicine and probiotics have a better effect in controlling and treating ovarian cancer. However, it demands further studies about different drugs, and probiotic strains. *L. plantarum* CLS can be used as adjunctive therapy with carboplatin through increased toxicity and changes in apoptosis gene expressions.

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