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Effect of Extracellular Vesicles Derived From Mesenchymal Stem Cells on K-562 Leukemia Cell Line

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Abstract

Background: Several studies show that mesenchymal stem cells (MSCs) possess anti-tumor properties, while other studies show that MSCs may promote cancer. Extracellular vesicles (EVs) are identified as novel mediators to communicate among cells, which may be used as vehicles to transfer the MSC information to the target cells.
Objectives: The current study aimed at investigating whether the human umbilical cord mesenchymal stem cell (HUSCMSC) derived extracellular vesicles (EVs) inhibits or promotes tumor cell growth in K562 chronic myelogenous leukemia cell line.
Methods: In the current experimental study, trypan blue exclusion and 3-(4, 5-dimethylthiazol-2-yl)-2, 5-diphenyltetrazolium bromide (MTT) assay were performed to investigate the cell viability and metabolic activity. Additionally, flow cytometry was employed to assess cell cycle and apoptosis of K562 cells after 72 hours exposure to EVs.
Results: The obtained results showed that no doses of EVs inhibited the tumor growth (mean ± standard deviation (SD) of cell viability at the day 10 was 94.4 ± 2.60, $P > 0.05$) and metabolic activity in three days (mean ± SD at the day 3 was 97.27 ± 2.46, $P > 0.05$). Furthermore, EVs also did not change the apoptosis rate (mean ± SD at the day 3 was 3.17 ± 2.34 for annexin positive cells, and 3.36 ± 1.91 for annexin/propidium iodide (PI) -positive cells, $P > 0.05$).
Conclusions: In conclusion, no significant changes in tumor cell growth were observed after treating K562 cells with MSCs-derived EVs.

Keywords: Cell Line, Extracellular Vesicles, Growth, K562 Cell, Leukemia, Mesenchymal Stem Cell, Tumor

1. Background

Mesenchymal stem cells (MSCs) are multi-potential stem cells residing in various tissues. Currently, they are widely studied due to their ability to differentiate between mesodermal ectodermic and endodermic lineages (1). Various studies show that MSCs support tumor growth (2, 3), while other studies reveal the anti-tumor effect of MSCs. MSCs isolated from various tissue such as human adipose (4), breast (5), and palatine tonsils (6) are able to interfere with cell proliferation, thereby blocking cancer cell cycle in G0/G1 phase. Cell contact between MSCs and cancer cells is not required for the biological activity of MSCs in vitro; instead, paracrine/soluble factors are responsible for the anti-tumor effect observed in the MSC-conditioned medium (7, 8).

Various studies focus on the effect of extracellular vesicles (EVs) on normal and tumor cells. EVs are released from a variety of cell types. EVs act as a vehicle for the intercellular exchanges capable of changing the behavior of the target cell, by interaction with the cell surface or transferring its content to the target cell. EVs contain lipids, proteins, and genetic materials. Transfer of these biological materials may cause important pathological and physiological changes in the target cells (1, 9, 10). It is shown that endothelial progenitors, liver stem cells, and MSCs-derived EVs can act as a vehicle to exchange functional mRNAs among cells that lead to the activation of regenerative programmes in differentiated cells both in vitro and in vivo (11-13). Recent studies show that MSCs-derived EVs (MSC-EVs) inhibit tumor growth in models of the Kaposi sarcoma (14), hepatoma cell line (hepG2) (15, 16), and ovarian cancer scov3 in vitro (17). EVs encompass multi-biological activities of their originating cells. Therefore, they are valuable objects for therapeutic purposes (9, 18).

Chronic myelogenous leukemia (CML) is a malignancy

arising from hematopoietic stem cells, characterized by a balanced genetic translocation (9, 19). Chemotherapy, bone marrow transplantation, and tyrosine kinase inhibitors, or combination of some of these therapies are currently applied; however, these methods may be curative only in some patients (20, 21).

2. Objectives

Several studies reported the effect of MSC-EVs on solid tumor growth, but the effect of MSC-EVs on leukemia is not well studied. The current study aimed at investigating whether MSC-EVs influence the growth of K562CML cell line. Therefore, the in vitro effect of MSC-EVs on cell proliferation, metabolic activity, and apoptosis of the K562 cell line was evaluated.

3. Methods

The current experimental study was conducted in Blood Transfusion Research Center, High Institute for Research and Education in Transfusion Medicine, Tehran, Iran in 2016. All the equipment was calibrated.

3.1. Cell Culture

CML cell line, K562, was purchased from ATCC and cultured in RPMI (Roswell Park Memorial Institute) 1640 (ATOCEL, Austria) containing 10% fetal bovine serum (FBS) (Gibco, USA) and 1% penicillin-streptomycin (Invitrogen, USA) at 37°C in a humidified atmosphere of 5% CO_2.

Human umbilical cord MSCs (HUCMSCs) were obtained from cord blood bank of the Iranian Blood Transfusion Organization and cultured in Dulbecco's modified Eagle medium low glucose (DMEM-LG) (ATOCEL, Austria) supplemented with 10% FBS and 1% penicillin-streptomycin.

3.2. Isolation of EVs

Extracellular vesicles (EVs) were obtained from the supernatant of MSCs (passage P3 - P5) cultured for 48 and 96 hours in DMEM-LG with 0.5% bovine serum albumin (BSA) (Roche, Switzerland). Briefly, the supernatant was centrifuged at 2000 g for 20 minutes two times in order to remove debris. Then, the supernatant was centrifuged at 20000 g for one hour at 4°C. The centrifuged pellet was resuspended in FBS free RPMI 1640 medium. The protein content of the resuspended pellet was measured through Bradford assay using the NanoDrop spectrophotometer (WPA, UK). Briefly, different dilutions of BSA were prepared (125, 250, 500, 1000, and 2000 μg/mL). Then, 10 μL BSA and EVs samples were mixed separately with 200 μL of Bradford solution (Sigma-Aldrich, USA) and optical density (OD) of the mixture was measured at 595 wavelengths. The standard curve was plotted by BSA samples and was applied to determine the protein concentration.

3.3. Characterization of MSC-EVs

Surface markers of the EVs were investigated using flow cytometry. Since EVs are derived from MSCs, they should possess MSC surface markers. Briefly, EVs were incubated with monoclonal antibodies conjugated with fluorescent dyes, including anti-CD34-FITC (fluorescein isothiocyanate), anti-CD44-FITC, anti-CD45-FITC, and anti-CD90-FITC for 30 minutes at 4°C. EVs were immediately analyzed with Partec CYFlow Space.

A particle-sizing instrument, Malvern Mastersizer 2000 laser diffraction system (Malvern Instruments Ltd., Worcestershire, UK) was used to determine the size distribution of vesicles by dynamic light scattering (DLS).

3.4. Viability Assay

K562 cells were cultured in the presence of MSC-EVs (0, 50, 100, 200, 500 μg/mL) for 10 days. Cell viability was evaluated using trypan blue (Sigma-Aldrich, USA).

3.5. Metabolic Activity

K562 cells (5000 cell/well) were treated with MSC-EVs (0, 50, 100, 200, 500 μg/mL) in a 96-well plate for 72 hours. Metabolic activity was evaluated by 3-(4,5-dimethylthiazol-2-yl)-2, 5-diphenyltetrazolium bromide (MTT) assay (Sigma-Aldrich, USA). MTT dye (0.5 mg/mL) was added to each well (test was performed in triplicates) and incubated at 37°C and 5% CO_2 for three to four hours. Throughout the post-incubation period, 100 μL dimethyl sulfoxide (DMSO) was added and the plate was kept at room temperature in darkness for 30 minutes. The absorbance of each well was spectrophotometrically measured at 570 nm using a microplate ELISA (enzyme-linked immunosorbent assay) reader. K562 cells cultured in fresh RPMI without EVs were used as controls. DMSO was used as blank. Cell metabolic activity rate was expressed as the absorbance ratio of treated cells to the control cells.

3.6. Annexin/PI Apoptosis Analysis

To investigate the effect of MSC-EVs on the apoptosis of K562, annexin/PI staining (BD Biosciences, USA) was performed. The cells (100×10^3 cell/well) were cultured in RPMI medium containing MSC-EVs (0, 500 μg/mL) in a six-well plate. After 72 hours, the cells were collected and washed with cold PBS twice; then, resuspended in binding buffer (1X). The cells were stained with 5 μL of FITC annexin V and 5 μL of PI, and incubated for 15 minutes at room temperature, followed by the analysis with Partec CYFlow Space. K562 cells cultured in RPMI without EVs were used as a control.

3.7. Cell Cycle Analysis

To evaluate the cell cycle, K562 cells were cultured with MSC-EVs (0, 500 μg/mL) for 72 hours. PI staining was used to detect DNA content of K562 cells by flow cytometry, according to the manufacturer's protocol. Briefly, cells were seeded in a six-well plate at the density of 100 cell/well and incubated in the presence of MSC-EVs (0, 500 μg/mL) for 72 hours. The cells were washed twice with PBS; then, 50 μL of RNase A stock solution and 500 μL of PI staining solution (eBioscience, USA) were added to the cell pellet and incubated for 15 minutes at 37°C. Finally, the cells were analyzed with Partec CYFlow Space.

3.8. Statistical Analysis

Statistical analysis was performed using the Shapiro-Wilk, parametric (*t*-test and ANOVA), and non-parametric (median) tests ($P < 0.05$ were considered significant).

4. Results

4.1. MSC-EVs Characterization

Flow cytometry analysis showed that MSC-derived EVs were positive for surface-expressed molecules CD90 and CD44, normally expressed by MSCs, and negative for hematopoietic stem cell surface molecules, CD34 and CD45 (Figure 1). The average size distribution of EVs measured by size distribution analysis was 340 nm (Figure 2).

4.2. Cell Viability

Trypan blue exclusion assay for 10 days showed that the viability of K562 cells treated with MSC-EVs remained unchanged. There was no significant difference in cell viability compared with the control cells (Figure 3) ($P > 0.05$).

4.3. Metabolic Activity

According to the MTT results presented in Figure 4, no significant differences were observed after 72 hours, compared with the control cells ($P > 0.05$).

4.4. Effect of MSC-EVs on the Apoptosis of K562 Cells

The apoptosis rate in K562 cells treated with EVs (500 μg/mL) for 72 hours did not change in comparison with the control cells (Figure 5) ($P > 0.05$).

4.5. Effect of MSC-EVs on the Cell Cycle

Cell cycle analysis was performed to assess the effect of MSC-EVs on K562 cells. SubG1 phase significantly decreased after 72 hours exposure to 500 μg/mL MSC-EVs as compared with control cells (Figure 6) ($P < 0.005$).

5. Discussion

EVs are described as mediators for cell-to-cell interactions that reprogram target cells through transfer of proteins, functional mRNAs, and miRs as well as activation of signaling pathways. In particular, it is suggested that they are involved in genetic changes since they can transfer genetic materials such as mRNAs and miRs (19, 22). Stem cells such as MSCs and progenitors release abundant EVs (23).

MSCs play a bilateral role in cancer progression. They secrete multiple bioactive factors to promote or inhibit tumor growth. Stimulatory and inhibitory effects of MSCs on the growth of cancer cells indicate a balance between anti- or pro-apoptotic factors (7, 24). Different studies unveiled the anti-tumor effect of MSCs such as the Kaposi sarcoma (14), breast cancer, and non-Hodgkin lymphoma (25, 26). In contrast, other studies demonstrated the supportive effect of MSCs on cancer progression. MSCs increase the growth rate in FMC-7 cell line, and the released paracrine factors of MSCs promote cell growth in breast cancer estrogen receptor α (ERα)-positive cell lines such as T47D (27). It is shown that no cell contact between MSCs and tumor cells is required for in vitro biological activity of MSCs.

In the current study, the effect of MSC-EVs on growth and apoptosis of K562 cell line was evaluated. No significant decrease in K562 cell growth and proliferation was observed after ten days of exposure to MSC-EVs. Moreover, no significant increase was observed in the K562 cell apoptosis after exposure to MSC-EVs.

The mechanism of effect of MSCs on tumor cells is not widely studied; nevertheless, many studies reported that MSCs can protect leukemic cells by interfering in apoptosis. Lin et al. (28) showed that the anti-apoptotic genes such as Bcl-XL are upregulated in U937 co-cultured with MSCs. In addition, the pro-survival factors released from MSCs are important to sustain the survival of the leukemic cells (28). Also, MSCs can induce drug resistance in leukemic cells (29).

EVs derived from cells can change tumor cell behavior by delivering functional mRNA and microRNA sequences; however, crucial markers in the tumor cells play an essential role in EVs function. Du et al. (30) showed that EVs released from human Wharton jelly mesenchymal stem cells (hWJ-MSCs) attenuated the growth of bladder tumor cells (T24); however, these EVs promoted human renal cell carcinoma (786 - 0) growth. Unlike T24, 786 - 0 cells express both HGF and c-MET, representing a cancer early progenitor cell marker (30, 31).

Several studies reported that tumor cells consistently secrete microvesicles. Tumor-derived microvesicles (TMVs) contain lipids, proteins, and nucleic acids. They can transfer bioactive content to the cells in the tumor microenvironment. They promote signaling responses in the surrounding cells and influence the tumor microenviron-

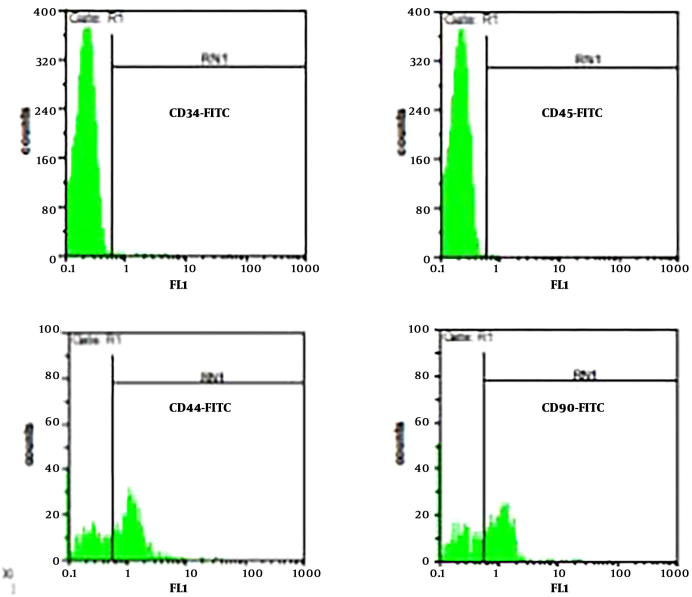

Figure 1. Flow cytometry plots; flow cytometry analysis showed that EVs derived from UC-MSC were positive for surface-expressed molecule, symbolically expressed by MSCs CD44 and CD90, and negative for surface-expressed molecule, symbolically expressed by hematopoietic cells CD34 and CD45.

Results

		Diam. (nm)	% Intensity	Width (nm)
Z-Average (d.nm): 636	**Peak 1:**	340	79.8	63.7
Pdl: 0.657	**Peak 2:**	93.9	20.2	15.8
Intercept: 0.925	**Peak 3:**	0.00	0.0	0.00

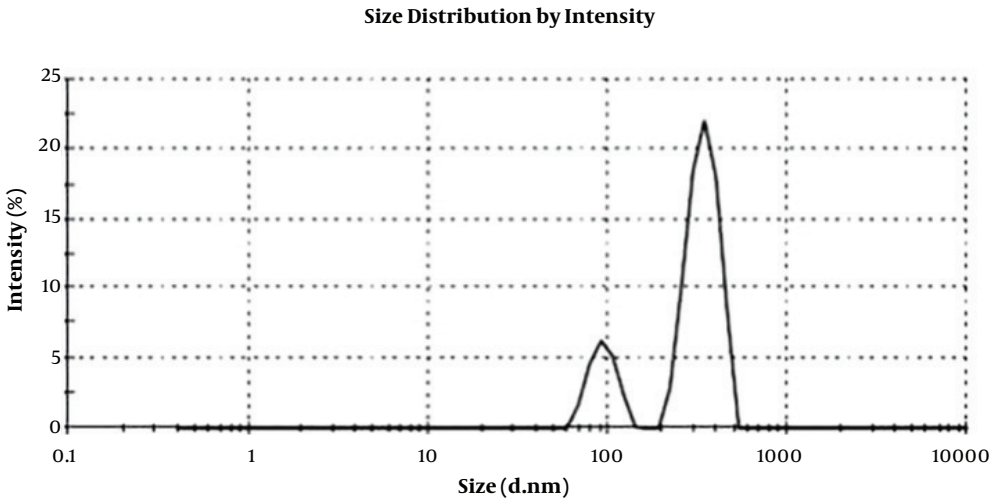

Figure 2. Size distribution of EVs derived from umbilical cord MSCs; the size distribution was determined to be in the range of 340 nm.

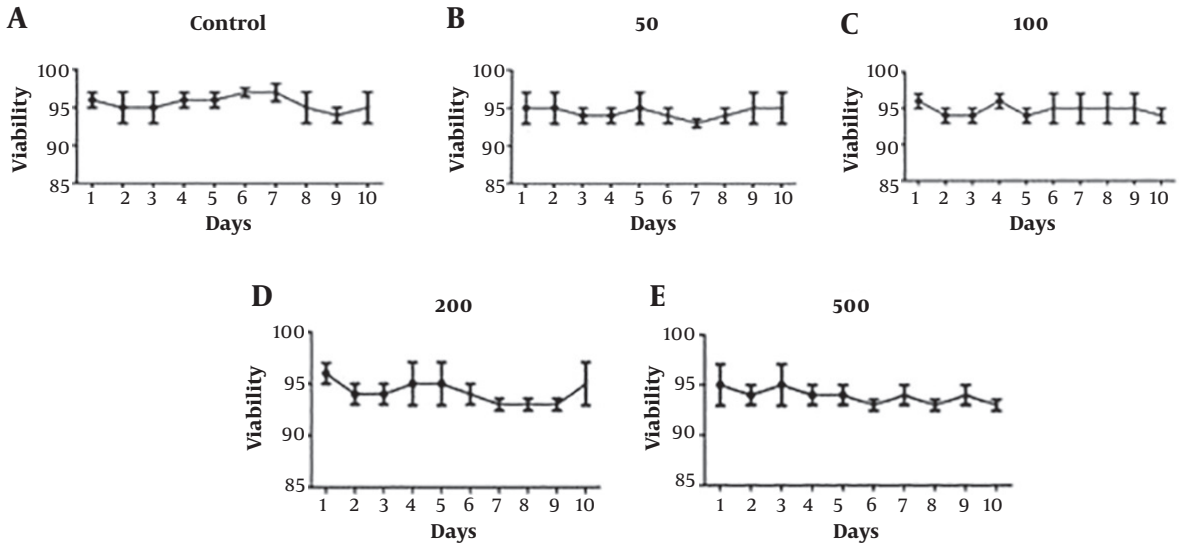

Figure 3. Cell viability assay; after 10 days of exposure to MSC-EVs (50, 100, 200, 500 μg/mL) no significant decrease in K562 cell viability (%) was observed compared with the control ($P > 0.05$).

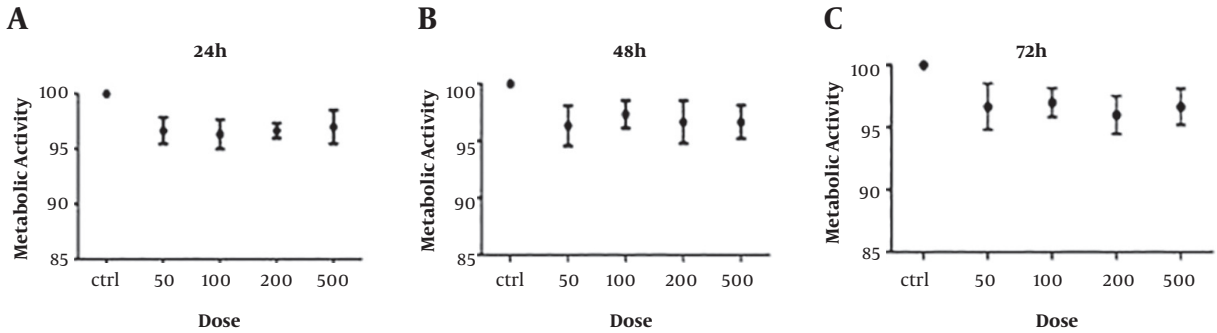

Figure 4. MTT assay analysis; K562 cells were cultured in the presence of MSC-EVs (50, 100, 200, 500 μg/mL) for 72 hours. No significant decrease in the metabolic activity (%) was observed ($P > 0.05$).

ment (32). TMVs alter the content and behavior of normal cells. They can change the phenotype of normal cells into malignant cells. TMVs promote cell growth via different mechanisms such as transferring oncogenic activities to other tumors and normal cells and drug resistance by sequestering and taking drugs out of the tumor cells (33). Tumor cells take up autologous MVs more than heterologous or normal microvesicles. Surprisingly, TMVs at a concentration as low as 0.1 μg/mL were able to significantly increase tumor invasion (34).

5.1. Conclusions

Various studies reported that MSCs play a dual role in cancer progression. Although it seems that MSCs are capable of treating cancers, a large number of studies support their involvement in leukemogenesis (29). In conclusion, the current study results showed no significant changes in tumor cell growth after exposing K562 cells to MSCs-EVs. Whereas the current study was conducted on the cell line, for better conclusions, it is recommended that similar studies be conducted on patient samples.

Acknowledgments

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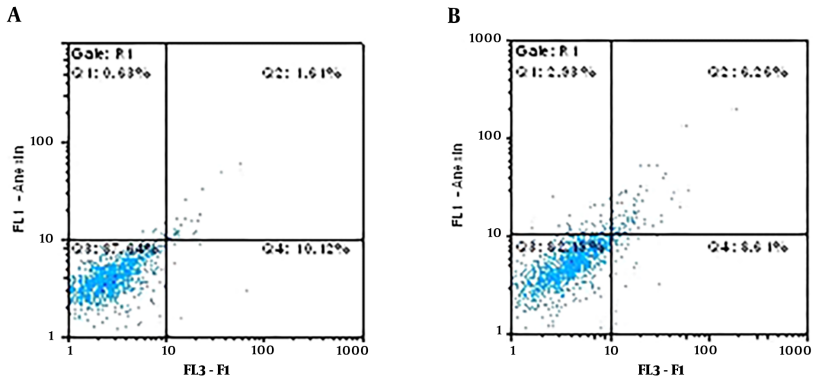

Figure 5. Apoptosis analysis; (A) control; K562 cells cultured in fresh RPMI without EVs, (B) K562 cells were treated with MSC-EVs for 72 hours. Apoptosis rate did not change after 72 hours exposure to MSC-EVs compared with the control ($P > 0.05$).

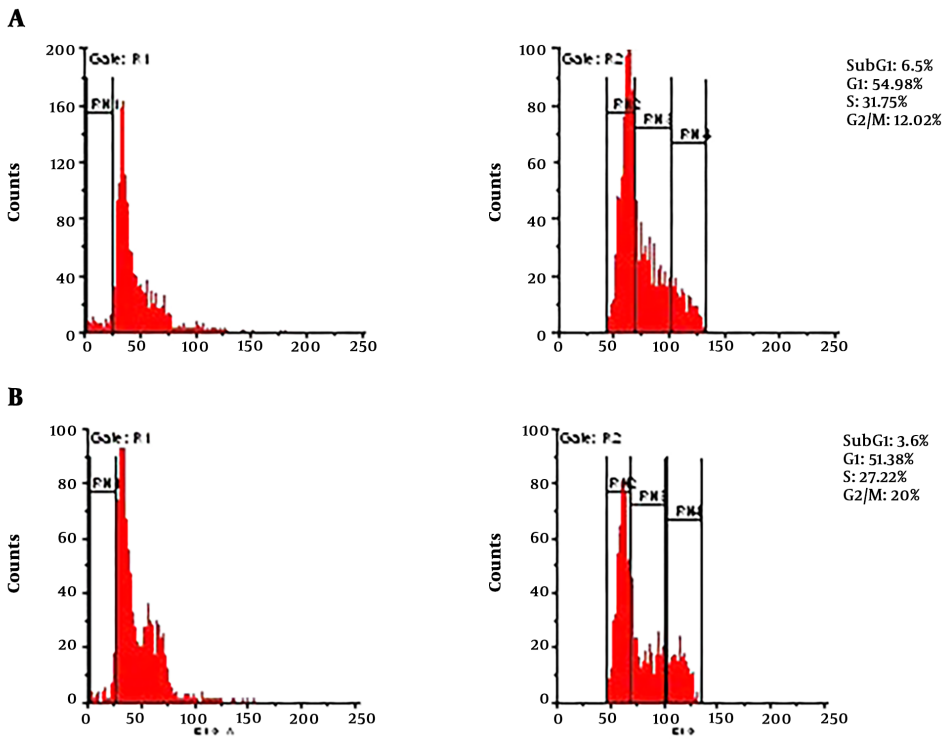

Figure 6. Cell cycle analysis; (A) K562 cells cultured in fresh RPMI without EVs were used as the control, (B) K562 cells were treated with MSC-EVs for 72 hours. SubG1 phase significantly decreased (P value = 0.005). RN1: subG1, RN2: G1, RN3: S, RN4: G2/M

Footnotes

Conflicts of Interest: The authors declared no conflict of interest.

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