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Original Article

Silver Nanoparticles Synthesised Using Sargassum Angustifolium Can Effect on MiR-25 and MiR-143 Expression in Acute Lymphoblastic Leukemia

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Abstract

Background: One of the treatments of acute lymphoblastic leukemia (ALL) is chemotherapy but it can destroy other normal cells as well as cancer cells, which can lead to infection and bleeding. The widespread consumption of nanoparticles synthesized biogenically by seaweed is because of their easy accessibility and usefulness. We aimed to investigate silver nanoparticles synthesised using Sargassum on the expression of miR-25 and 143 in Jurkat cell line.

Methods: In an interventional study in 2019, Bushehr, Iran, following culture of the Jurkat cell line with 95% survival rate, we applied different drug concentrations to 20,000 cell lines and control cells separately to determine maximum cell growth inhibition and IC50. After treatment for 48 h, RNA extraction was performed, and Real Time PCR was used to evaluate miR-25 and 143 expressions.

Results: MiR-25 expression in the groups that treated with the maximum dose and IC50 of silver nanoparticles and algae extract was decline but did not differ significantly from that of the control group. Conversely, miR-143 showed a remarkable decline in both treatment groups ($P<0.0001$). After treating PBMC with nanoparticles the expression level of miR-25 and miR-143were not significantly different.

Conclusion: The level of miR-25 and 143 expressions were decrease in the IC50 dosage of silver nanoparticles and algae extract produced. The level of miR-25 expression decreased more in the treated Jurkat cell line than the level of miR-143 expression in normal lymphocytes. Given the oncogenicity of miR-25 in the Jurkat cell line, it is suggested that decreasing level of this microRNA can help to apoptosis of leukemic cells.

Keywords: Acute lymphoblastic leukemia (ALL); Nanoparticles; Silver

Introduction

Acute lymphoblastic leukemia (ALL) is the most common malignancy that lymphoid precursors proliferated in the bone marrow, peripheral blood, and extra-medullary areas (1). Which is the most common malignancy among children, especially in Western countries (2) and accounts for

almost 25% of all children's cancers (3). T-ALL can see in pediatric and adult cases of ALL in Europe-US and Japan in men as women (4). The treatment protocol for the disease is 2 to 3 years of chemotherapy that is given to patients during 3 phases of maintenance, induction and consolidation. However, in addition of cancer cells, other normal cells can destroy with chemotherapy that can lead to infection and bleeding (5). In confronting with these clinical challenges, many efforts are being made to decipher the T-ALL molecular event codes to identify more definite therapeutic targets and to develop more real anti-leukemic treatments with lower toxicity and drug combinations (4).

MicroRNAs are little, regulatory, non-coding, single-stranded RNAs of 19-24 base in distance that negatively regulate translation in the pre-expression stage of the gene and target gene expression by targeting specific mRNAs (6-8). Approximately fifty percentages of the miRNA genes are sited in the cancer-related gene region or in the delicate gene region, and some of them are directly involved in the spread and progression of cancer. Expression profiles of microRNAs classify tumors based on their proliferation and differentiation (6,9). MicroRNAs are potentially oncogenes or tumor suppressor genes that are occupied in tumor spread and progression. MicroRNAs are involved in a number of tumors, involving the pancreas, colon, stomach, breast, liver, lung, and nasopharyngeal canal (nose and throat) (10). In cancer cell line Jurkat, miR-25 affects the cell by regulating the expression of target genes. Among the target genes of this microRNA, according to a search in the miRmap database, is MDM2, which is an anti-apoptotic gene and binds to P-53 to prevent its function (11) and the EphA8 gene, which directly targets 3′UTR-EphA8, and overexpression of miR-25 inhibits EphA8 expression, resulting in the absence of EphA8, proliferation, movement, and aggression ALL cells occur in-vitro (12). ERK5 is an exact target of miR-143, this miRNA is a tumor suppressor and apoptotic miR that increasing expression of this miR can decrease ERK5. MiR-143 can induce apoptosis through fas by targeting the ERK5 gene (13).

Nanoparticles of oxidizing metals are used in the field of biosensors and diagnostic instruments, catalysts, anticancer and antimicrobial agents due to their physical and chemical properties. Synthesis of nanoparticles by seaweed is used because of its simple access and usefulness (14). Seaweeds are a group of marine plants that are a basis of organically active materials for medical applications. Therefore, the biosynthesis of nanoparticles using seaweed has moved our attention to the use of renewable marine resources. Traditionally; silver metal has been manipulated as a therapeutic agent in the care of diseases. In addition, silver salt solution has more benefits in epilepsy, nicotine habit, gastroenteritis, stomatitis and sexually transmitted diseases. As a result, synthesized silver nanoparticles play a favorable role in medical requests (15). *Sargassum Angostifolium* is a brown algae and has beneficial properties such as antioxidant, antibacterial, immune system stimulant as well as acetylcholinesterase (ACHEI) activity, which invites researchers to investigate the synthesis of silver nanoparticles using it (16).

Therefore, we decided to study the influence of silver nanoparticles with sargassum brown seaweed extract on miR-25 and miR-143 in the cell line of Jurkat and to measure the expression of these microRNAs in healthy and juvenile cells after exposure to nanoparticles.

Materials and Methods

The study was approved at Bushehr University of Medical Sciences (IR.BPUMS.REC.1398.014).

Synthesis of silver nanoparticles

Brown seaweed extract with silver nanoparticles was obtained from the Iranian National Institute for Oceanography and Atmospheric Science, in 2019, Bushehr, Iran. That was synthesis according to its protocol (16).

Cell culture

T-ALL cell line (Jurkat cell lines) from National Cell Bank of Iran (Pasteur institute of Iran) were cultured over a period of 2 months in RPMI-1640 (Gibco/USA) (culture medium) with 2 mM/L-glutamine (Sigma-Aldrich, MO, USA) complemented with antibiotic (penicillin/streptomycin)(Invitrogen, Life Technologies, Camarillo, CA, USA) and 10% FBS (Life Technologies, Camarillo, CA, USA). All cells were incubated in a humidified atmosphere, containing 5% CO2 at 37 °C on plastic petri dishes. For detection of the percentage of living cells, trypan blue (Gibco/USA) staining was used at 0.4%. For the study, cellularity of a cell flask was more than 95%.

MTT assay

The Neubauer slide was utilized to count cells, including 2× 104 cells per well of Jurkat cell line and peripheral blood mononuclear cells (PBMC). Following this, the cells were transferred onto a 96-well plate. The experimental medium replaced the previous media after 24 h, which contained varying dilutions of silver nanoparticles synthesized using Sargassum (1, 2, 4, 8, 16, 32, 64, and 128) for evaluating the effect of the nanoparticle. Control group received RPMI only. The initial dose of the main drug, brown seaweed extract with silver nanoparticles, was 100.17 mg/ml, and the initial dose of brown seaweed extract and silver nitrate (Prepared by Bushehr Oceanographic Center (14) was 100 mg/ml and 170 ng/ml, respectively. Cell viability was evaluated using the MTT assay following standard protocols at 24, 48, and 72 h. 10 µL MTT was added to each well, at 24 h and for 4 h, the plates were incubated at 37 °C. Subsequently, Formosan crystals were solubilized in dimethyl sulfide (DMSO, Bioscience, USA, 100 µl/well). A blank control containing only culture medium was used for comparison. Optical density (OD) was calculated with a microplate reader at 570 nm. All experiments were triply repeated. The percentage of cytotoxicity was measured by the following equation using OD:

Cytotoxicity = [(OD Test**-OD**Blank**) - (OD-**Control**) ×100]/ (OD**Control**)**

RNA Extraction and cDNA Synthesis

After 48 h treatment of Jurkat cell line and normal PBMC the RNA of these cells were extracted by Total RNA Isolation Kit (Santa Cruz Biotechnology, Inc., Texas, USA). The concentration and purity of RNA were suitable at 260 to 280 and 230 to 260 wavelengths. A standard RNA (Ladder) was used as the measurement criterion. Polyadenylation was performed for cDNA synthesis; this was incubated for 30 min at 37 °C then placed at 65 °C for 20 min. The cDNA synthesis step takes place immediately after polyadenylation. At this stage, 10 microliters of polyadenylated RNA were poured into each microtube with one microliter of BON-RT (Bon Yakhteh Company, Iran) adapter primer (10µM) and the volume of each microtube was increased to 13 microliters with DEPC (Diethylprocarbonate) water. Then we put it in a dry bath for 5 min at 75 °C. The tubes were placed on ice and 7 µl of RT enzyme, dNTP mix (100 mM) and 5x RT buffer were added to each microtube. After placement in specific time cycles, the synthesized cDNAs were finally examined for concentration and purity with nanodrap. Then cDNAs stored in -20 °C until Quantitative Real-Time RT-PCR (qRT-PCR) was done.

*Quantitative Real-Time RT-PCR (*qRT-PCR)

Quantitative Real Time RT-PCR of miRNA was executed following the guidelines from the manufacturer. (Yekta Tajhiz Azma, Iran). The sequence of primers (Bon Yakhteh company, Iran) used is arranged in Table 1. Materials used for this test are 0.1 ml qPCR microtubes (Bioplastics, USA), 0.2 ml microtubes (Bioplas lab, USA), Master Mix (Yekta Tajhiz Azma, Tehran, Iran), DEPC water (Sina Gene, Iran), crystalline and yellow head sampler (QClab company, China), sampler (NICHIRYO company, Japan), microfusion / spin machine (Amitis Gene company, Iran), machine (Applied Biosystems, Foster City, CA, USA).

Table 1: Quantitative Real Time RT-PCR forward and reverse primers

Gene	Primer sequences
Forward miR-25	5′-CATCGCACTTGTCTTG-3′
Forward miR-143	5′-ACTGTTGAGATGAAGCAC-3′
Forward miR -17	5′-GAGCCAAAGTGCTTACAGTGC-3′
Universal Reverse	5′-AGTGCAGGGTCCGAGGTATT-3′

MiR-17 is an internal control for miRNA expression for real-time RT-PCR. Y.R. miR-17, miR-107, and miR-103 are the best candidates for internal controls for measuring of miRNA expression (17).

Statistical analysis

Statistical analysis was done with SPSS-22 software (IBM Corp., Armonk, NY, USA) and GraphPad Prism 7) GraphPad Software, Inc., CA, USA). Quantitative values of all experiments were stated as the mean ± Standard Deviation (SD) for comparing between groups. Differences between sample groups were examined with one-way ANOVA and used for data analysis and charting and P value less than 0.05 was measured as a significant difference. After a significant ANOVA, the dunnett's post hoc test used to determine which differences were significant.

Results

Viability of Jurkat Cell Line

Viability of jurkat cell line that treated with silver nanoparticles synthesised using Sargassum's was examined by MTT assay. After treatment with 12.5 mg/ml silver nanoparticles synthesised using Sargassum, more than 99% of Jurkat cells dead, and the IC50 of this nanoparticle was 100.17 mg/ml in Jurkat cell line (Fig. 1).

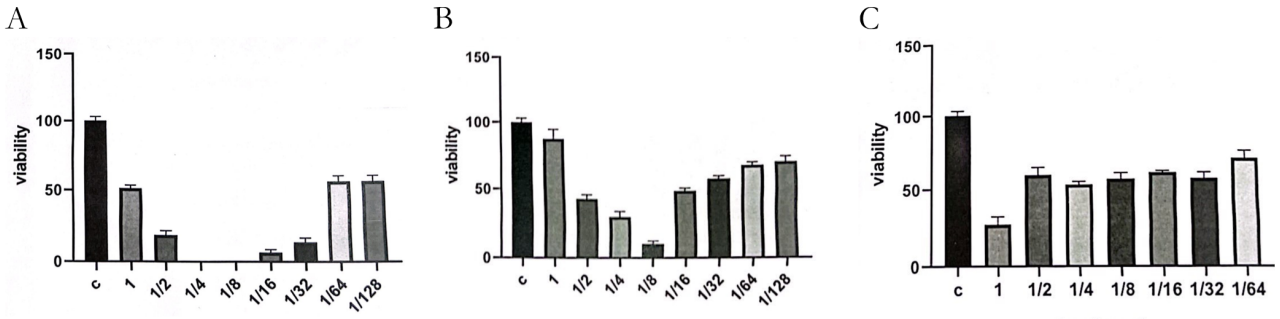

Fig. 1: The MTT assay. A) Treatment with dilutions of Silver Nanoparticles. The initial dose of the main drug, brown seaweed extract with silver nanoparticles, was 100.17 mg/ml B) Treatment with dilutions of Sargassum. The initial dose of brown seaweed extract was 100 mg/ml C) Treatment with dilutions of silver nitrate, The initial dose of silver nitrate 170 ng/ml

The level of miR-25 expression decreased in Jurkat Cell Line

To evaluate the level of miR-25 expression in the Jurkat cell line that treated with the maximum dose and 12.5 mg/ml and 100.17 mg/ml nanoparticles, silver nitrate and brown seaweed, we ran real-time PCR analysis. As depicted in Table 2 and Fig. 2A, our data exhibited the level of miR-25 expression compared to the control were not significantly different and decreased, respectively.

Table 2: Changes in miR-25 expression level in Jurkat Cell Line and PBMCs compare to untreated cells.

Variable	Fold changes in Jurkat	*P* value	Fold changes in PBMC	*P*-value
Nano-Max	0.7225	0.7153	9.4479	0.1189
Nano-50	0.0011	<0.0001	0.0026	0.0006
Ag-Max	0.6912	0.6786	0.0004	<0.0001
Ag-50	0.2138	0.0980	0.0305	0.0213
Algae-Max	0.5542	0.5098	0.0167	0.0087
Algae-50	0.0144	0.0002	0.3647	0.4690

Nano-Max: cell treated with the maximum dose nanoparticles, Nano- 50: cell treated with the IC50 nanoparticles, Ag- Max: cell treated with the maximum dose silver nitrate, Ag- 50: cell treated with the IC50 silver nitrate, Algae-Max: cell treated with the maximum dose Sargassum, Algae- 50: cell treated with the IC50 Sargassum

The level of miR-143 expression decreased in Jurkat Cell Line

Examination of miR-143 in the Jurkat cell line treated with the maximum dose and 100.17 mg/ml silver nitrate displayed that the expression level of this miRNA was not significantly different and in the jurkat that gave the maximum and IC50 dose of nanoparticles and brown seaweed compared to the control decreased, respectively (Table 3 and Fig. 2B).

Table 3: Changes in miR-143 expression level in Jurkat Cell Line and PBMCs compare to untreated cells

Variable	Fold changes in Jurkat	*P*-value	Fold changes in PBMC	*P*-value
Nano-Max	0.0005	<0.0001	0.5473	0.6047
Nano-50	0.0014	<0.0001	0.0015	<0.0001
Ag-Max	1.4278	0.6760	0.0006	<0.0001
Ag-50	0.2482	0.1163	0.0174	0.0029
Algae-Max	1.8477	0.4738	0.0039	0.0002
Algae-50	0.1264	0.0258	0.0245	0.0053

Nano-Max: cell treated with the maximum dose nanoparticles, Nano- 50: cell treated with the IC50 nanoparticles, Ag- Max: cell treated with the maximum dose silver nitrate, Ag- 50: cell treated with the IC50 silver nitrate, Algae-Max: cell treated with the maximum dose Sargassum, Algae- 50: cell treated with the IC50 Sargassum

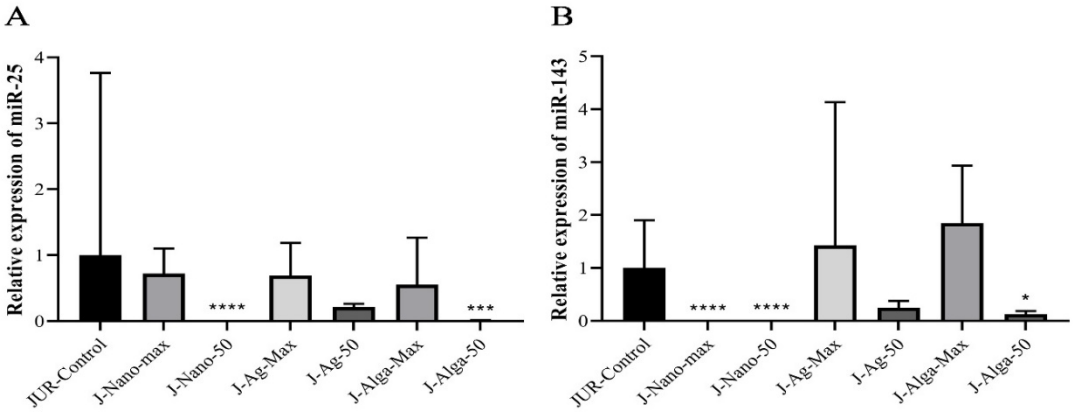

Fig. 2: A) MiR-25 expression level in Jurkat Cell Line compare to untreated cells. (**** *P*-value <0.0001 and *** *P*-value <0.0002). B) MiR-143 expression level in Jurkat Cell Line compare to untreated cells. (****P-value<0.0001 and * *P*-value<0.05). JUR-Control: Jurkat cell line without treatment, J-Nano-Max: Jurkat cell line treated with the maximum dose nanoparticles, J-Nano- 50: Jurkat cell line treated with the IC50 nanoparticles, J-Ag- Max: Jurkat cell line treated with the maximum dose silver nitrate, J-Ag- 50: Jurkat cell line treated with the IC50 silver nitrate, J-Algae- Max: Jurkat cell line treated with the maximum dose Sargassum, J-Algae- 50: Jurkat cell line treated with the IC50 Sargassum

The level of miR-25 expression decreased in PBMC

When normal lymphocyte group was treated with the maximum dose nanoparticles in 48 h, compared to the control was not significantly different and in the PBMC group treated with the IC50 dose nanoparticles and maximum dose and IC50 of silver nitrate and brown seaweed compared to the control there was a noticeable decrease in miR-25 expression, respectively (Table 2 and Fig. 3A).

The level of miR-143 expression decreased in PBMC

Mi-143 expression has a same decreasing level after treating with the IC50 dose nanoparticles and maximum dose and IC50 of silver nitrate and brown seaweed compared to the control, respectively and examination of this miRNA in PBMC group treated with the maximum dose of nanoparticles paralleled to the control showed that the expression level of this miRNA statistically not significantly different (Table 3 and Fig. 3B).

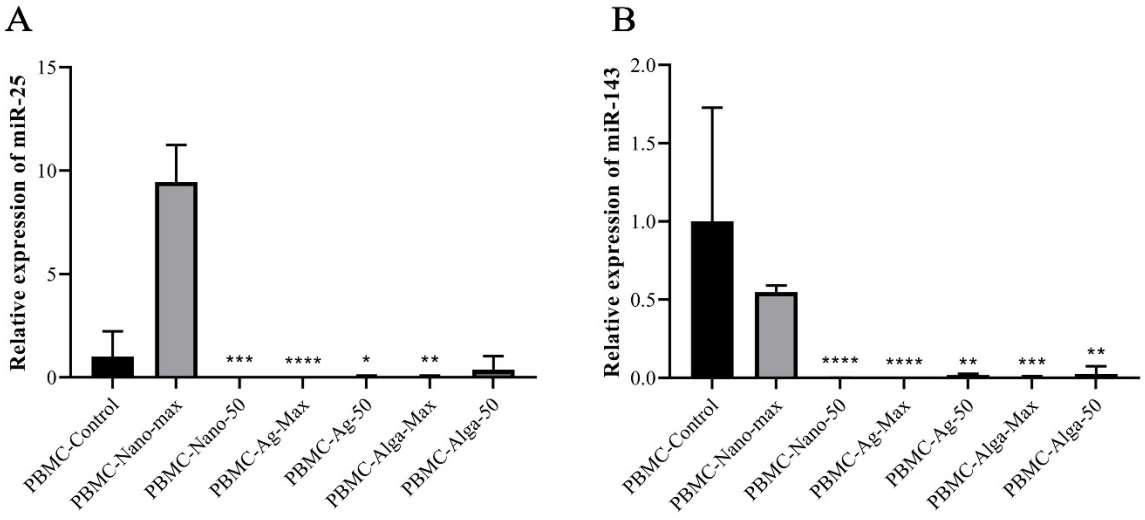

Fig. 3: A) MiR-25 expression level in PBMC compare to untreated cells. (****P-value<0.0001, ***P-value<0.0006, **P-value<0.0087, and *P-value<0.05). B) MiR-143 expression level in PBMC compare to untreated cells. (**** P <0.0001 and *** P <0.0002 and ** P <0.0053). PBMC-Control: PBMC without treatment, PBMC -Nano-Max: PBMC treated with the maximum dose nanoparticles, PBMC -Nano- 50: PBMC treated with the IC50 nanoparticles, PBMC -Ag- Max: PBMC treated with the maximum dose silver nitrate, PBMC -Ag- 50: PBMC treated with the IC50 silver nitrate, PBMC -Algae- Max: PBMC treated with the maximum dose Sargassum, PBMC -Algae- 50: PBMC treated with the IC50 Sargassum

Evaluation of miR-25 expression changes in Jurkat Cell Line compared to PBMC group

The level of miR-25 expression in the normal lymphocyte compared to the control Jurkat were not significantly different (P>0.05).

Changes in the miR-25 expression in the PBMC group treated with maximum dose and IC50 nanoparticles compared to the Jurkat group that treated with maximum dose and IC50 nanoparticles were related with a decrease of 13 times (P=0.0437) and no significant difference (P>0.05), respectively. Changes in the expression of this miRNA in the PBMC group treated with the maximum dose and IC50 of silver nitrate compared to the Jurkat group treated with the maximum dose and IC50 of silver nitrate were related with a 1584-fold increase(P<0.0001) in expression and no significant difference (P>0.05), respectively. Changes in the expression of this miRNA in the PBMC group treated with the

maximum dose and IC50 of brown seaweed compared to the Jurkat group treated with the maximum dose and IC50 of brown seaweed were associated with a 33.1285-fold increase (P=0.0075) in expression and decrease of 25.25 times (P=0.0128), respectively.

Evaluation of miR-143 expression changes in Jurkat Cell Line compared to PBMC group

Changes in miR-143 expression were not significantly different in the normal control lymphocyte group compared to the control Jurkat group (P>0.05). Changing of miR-143 expression in the PBMC group treated with maximum dose and IC50 nanoparticles compared to Jurkat groups treated with the same doses of nanoparticles were associated with a decrease of 1000-fold (P<0.0001) and no significant difference (P>0.05), respectively. Changes in the expression of this miRNA in the PBMC group treated with the maximum dose of silver nitrate compared to the Jurkat group treated with the maximum dose and IC50 of silver nitrate were associated with an increase in expression of 23170 times (P<0.0001) and an increase of expression of 14.58 times (P=0.0188), respectively. Changes in the expression of this miRNA in the group PBMC treated with maximum dose and IC50 of brown seaweed compared to the Jurkat group of treated with the same doses of brown seaweed were associated with 469.187 expression increase (P<0.0001) without significant differences (P>0.05), respectively.

Discussion

In the Jurkat cell line that treated with the maximum and IC50 dose of silver nanoparticles groups and algae extract, the level of miR-25 expression was not significantly different from the control and decreased by 1000-fold, respectively, while that for miR-143 decreased by 1000-fold, respectively and a 714-fold decrease. The level of miR-25 and miR-143 expression was not significantly different from jurkat cell line that treated with silver nitrate or Sargassum and untreated cells. In previous studies, silver nanoparticles synthesized from seaweed extract had anti-cancer, antioxidant, antibacterial, antitumor, biological activity as well as high cytotoxic activity (18-22). MiR-25 has been found to exhibit oncogenic properties in cholangiocarcinoma, Hela cells, and gastric cancer. In contrast, reports indicate that miR-25 may function as a tumor suppressor in colorectal cancer and anaplastic thyroid cancer. Oncogene or tumor suppressor of miR-25 in the body depends on the specific tissue type (23). Enhanced expression of miR-143 is related with cessation of cell growth. Moreover, the expression of miR-143 is significantly reduced in prostate cancer and by changing the level of this miRNA epression, this cancer can treat. Reduced levels of miR-143 in cancerous cells can directly induce carcinogenesis. MiR-143 functions as a tumor suppressor and is a crucial diagnostic indication in prostate cancer (6). MicroRNAs can affect significantly in breast tumorigenesis. One of this miRNAs is miR-143 that is usually significantly suppressed in breast cancer (24) and there is a decrease in expression in breast cancer tissue compared to normal breast tissue. Increased expression inhibits the proliferation of cancer cells (25).

Many materials were produced by marine organisms such as terpenoids, bromophenols, carotene, phlorotannins, sulfated polysaccharides, steroids, and halogenated compounds (26). Using the compounds that produced from marine sources has expanded much notice. Different materials with involving biological and physiological activities produced from Seaweed algae and have antitumor activity, used instead of current cancer therapies (27). Fucoidan taken from algae have different act such as anti-tumor, anti-inflammatory, anti-viral, antimicrobial, wound healing, and antioxidant qualities have been previously confirmed (28). Both fucoidans extracted from Sargassum Angustifolium and Cystoseira Indica were anticoagulants activity and increased the PPT rate and can be instead of heparin in patients with high risk of coagulation disorders to increased clotting rate (29). Cell viability was decreased in cancer cells by Sargassum Angustifolium (30). Seaweed drugs (Brown algae) that ex-

tracted from algae displayed that antitumor activity (31).

Synthesis of Silver nanoparticle can play the role of new production of anticancer therapy. From many sources, namely, microorganisms and plants can produce nanoparticles (25). Obeidi and et al, displayed that silver nanoparticles synthesized could be used to treat ALL and reduce lymphoblast cells by inhibiting the expression level of the *IP3R3* and *GSK* genes. Then growth of lymphoblast cells inhibited (32).

Overexpression of miR-143 can effect on ERK5 gene and induce apoptosis by increasing Ly6G, the granulocyte surface markers (33,34).

Angostifolium is brown algae that have beneficial properties such as antioxidant, antibacterial, immune stimulant as well as acetylcholinesterase activity (ACHEI) (16). Based on the analysis of the results of MTT test with maximum dose and IC50, each of the extracts on normal lymphocytes of the body and fibroblast cells, all three extracts had a less lethal effect on normal lymphocytes and treated fibroblasts than the same untreated cells and Jurkat cells. In this study, the impact of silver nanoparticles and sargassum brown seaweed extract on Jurkat cancer cells was evaluated in this study, demonstrating a potential decrease in miR-25 and 143 expressions when utilizing the extract at a dose of IC50.

Conclusion

The silver nanoparticles with seaweed brown algae extract were examined for effecting on the level of miR-25 and miR-143 expression in the Jurkat cell line at maximum and IC50 doses and the results showed that IC50 dose of nanoparticles of silver and extract Algae had a decreasing effect on the expression of both miR-25 and 143. The level of miR-25 expression in treated Jurkat cell line relative to normal lymphocytes decreased more than the expression of miR-143.

Journalism Ethics considerations

Ethical issues (Including plagiarism, informed consent, misconduct, data fabrication and/or falsification, double publication and/or submission, redundancy, etc.) have been completely observed by the authors.

Availability of data and materials

All data created during this study are comprised in this published article.

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Conflict of Interest

The authors declare that there is no conflict of interests.

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