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ORIGINAL ARTICLE

The role of *Spirulina platensis* on the proliferation of rat bone marrow-derived mesenchymal stem cells

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ABSTRACT

Background and Objectives: *Spirulina platensis* micro-algae have some effects on cellular procedures. The proliferative potential of mesenchymal stem cells (MSCs) will be decreased after repetitive passage.
Materials and Methods: The stromal cells were isolated, and then proven by differentiating to adipogenesis and osteoblastic lineage. The cell markers such as CD90 and CD105 were detected by flowcytometry. MSCs were treated with extract of *S. platensis* in logarithmic concentrations. MTT and ATP assays were done to determine cell proliferation capacity. The antioxidant and antimicrobial properties of the extract were evaluated.
Results: The results obtained from differentiation confirm cells' potential for osteoblastic and adipoblastic differentiation. Detection of CD90 and CD105 markers over 70% proved that the majority of cells are MSCs. Statistical analyzes revealed a significant increase in MSCs proliferation in the concentration of 0.9 µl/ml *S. platensis*. DPPH assay demonstrated that the extract could scavenge free radicals up to 57%. Additionally, the extract showed the inhibition zone up to 11 mm against a different strain of bacteria by agar well diffusion assay.
Conclusion: Secreting nutritional elements, *S. platensis* extract can be used as an antioxidant, antimicrobial, and growth agent for enhancing the proliferation of MSCs. Furthermore, the optimum concentration for cell treatment with *S. platensis*'s extract was investigated.

Keywords: *Spirulina platensis*; Mesenchymal stem cells; Cell proliferation; Antioxidant; Antibacterial agents

INTRODUCTION

Spirulina is a green-blue microalga of the cyanobacterial family. This alga is mainly composed of two species: *Arthrospira platensis* and *Arthrospira maxima*. The microscopic appearance of *Spirulina platensis* is in the form of long spiral threads, thin and blue-green. Among the applications of this algae is its use as a supplement in agricultural industries (1). *S. platensis* comprises 51-71% protein that can supply many essential amino acids for the body (2). In addition, it has contained about 7% lipids which are rich in gamma-linolenic acid (GLA) (3, 4) to produce alpha-linolenic acid (ALA), linoleic acid (LA),

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stearidonic acid (SDA), eicosapentaenoic acid (EPA), docosahexaenoic acid (DHA) and arachidonic acid (AA) (5-7). *S. platensis* contains vitamins such as B1 (thiamine), B2 (riboflavin), B3 (nicotinamide), B6 (pyridoxine), B9 (folic acid), vitamin C, vitamin A and vitamin E (6, 7). Because of essential amino acids and vitamins, *S. platensis* can be applied as a supplement to cellular nutrition. Studies conducted in 2016 demonstrated that probiotics could affect various cells in different ways (8). Harper et al. (2003), studied the effect of *Lactobacillus acidophilus* on angiogenesis and macrophage activity stimulation. They depicted the effects of *L. acidophilus* on the chemotactic activity of leukocytes in vitro and the increase of fibroblasts in vivo, the proliferation of endothelial and inflammatory cells (9). Li et al. (2005) also assessed the impact of supernatant of a species of probiotic bacteria -*L. acidophilus*- on embryonic cells. The results obtained from this study showed the proliferation of a wide range of cells (10). Stem cells (SCs) are self-renewal cells that differentiate into various cell lineages (11-13). These cells are named Colony-forming fibroblastic cells, Mesenchymal Progenitor Cells, and Bone marrow Stromal cells (14-16). Two types of pluripotent stem cells located in the bone marrow are Hematopoietic Stem Cells (HSCs) and Stromal Cells (17). Nowadays, bone marrow mesenchymal stem cells (BMSCs) are applied to treat various diseases (18). One of the limitations of using primary cells is the limitation in the number of cell passages. Because in successive passages, the proliferative power of the cells is significantly reduced and the cells will move towards cell differentiation. Past studies have shown that cell proliferation depends on the environment and that extracellular signals can serve as a guide to cellular fate (19). In the present study, *spirulina* microalgae extract was used to evaluate the antioxidant and antibacterial properties and to evaluate their effects on the proliferation of mesenchymal stem cells isolated from rat bone marrow.

MATERIALS AND METHODS

Preparation of *Spirulina platensis*. Indonesian *Spirulina platensis* was obtained from the Science and Technology Park of Qeshm Island, Iran. It was cultured in Zarrouks and Conway medium. In short, samples were gently washed in the seawater, rinsed off in distilled water, and dried with a paper layer. After that, it was frozen at -20°C and then was lyophilized for storing. For later assessments, the dried substrate was then mechanically powdered in a mortar containing a silica gel desiccator at room temperature away from light (20).

***Spirulina cultivation*.** 50 ml of cultured *S. platensis* at the middle of the logarithmic growth phase and 2 ml sterile seawater enriched with Conway nutrient solutions (21) were poured in a borosilicate flask. Then they were incubated at room temperature for 30 minutes. All samples were divided into four groups, and the groups were exposed to a fluorescent lamp under 300 µmol photons $m^{-2}s^{-1}$ from the bottom at 12:12: dark-bright cycles. The average temperature was 23 ± 1°C for bright and 20 ± 1°C for dark periods. The salinity of medium was about 32%. Daily growth rate was measured by a microscopic cell counter. Daily culture pH was adjusted, too. The culture was concentrated by centrifugation at 7000 g at 15°C for ten minimums (at least once). After the final centrifugation, the cells were washed with free nitrogen and phosphorus by synthetic seawater. The degree of salinity to remove excess nitrogen from the medium was about 15%. The supernatant was thoroughly mixed, and then the number of cells was recounted to determine the percentage of dead cells. The cell mass was frozen at -20°C and then was lyophilized, and finally was stored at desiccator at room temperature away from light until further assessments (22).

Diphenyl-1-picrylhydrazine radical scavenging activity. This parameter was measured by the Zhang method with slight modification (23). First, 100 µl of extract at different concentrations were mixed with 100 µl of 0.16 mM DPPH solution. The samples were vortexed for one minute and were incubated in the dark for 30 minutes. Then their absorption was measured at 517 nm wavelength by a microplate reader (Polarstar Omega, Ottenberg, Germany). The radical scavenging capacity was calculated using the following equation (23) (1):

$$\text{Inhibition (\%)} = \left(\frac{S_{control} - (S_{sample} - S_{blank})}{S_{control}}\right) \times 100 \quad (1)$$

Where the $S_{control}$ is the absorbance of DPPH without sample, the S_{sample} is the absorbance of the extract

and DPPH solution, and the S_{blank} is the absorbance of the samples without DPPH solution.

***In vitro* antibacterial activity (Agar bioassay).** The antibacterial activity of different concentrations of microalgae extracts against Gram-negative and Gram-positive bacterium (*Escherichia coli* PTCC No: 1533, *Salmonella typhimurium* PTCC No: 1609, *Bacillus subtilis* PTCC No: 1156, *Staphylococcus aureus* PTCC No: 1917 obtained from Iranian research organization for science and technology, Tehran, Iran) was calculated by agar well diffusion assay. 25 ml of the mixture of adjusted medium with Muller Hinton Agar (MHA) was poured into Petri dishes. They were kept at 27°C for 30 minutes to solidify. Each plate was marked for the organisms inoculated. With the help of a sterile borer, the plates were punched separately, and a well with a diameter of 6 mm was created. After adding 100 ml of microalgae extract to each well, the plates were incubated at 37°C overnight. The zone of inhibitions was calculated in millimeters. The result was repeated three times (24).

Isolation and culture of rat bone marrow-derived mesenchymal stem cells. The rat bone marrow mesenchymal stem cells (rBMSCs) were isolated by the flushing method by slight modifications (25). Briefly, a six-week-old rat with approximately 300 gr weight was purchased from Pasteur Institute of Iran, Tehran. Asphyxiation was done by CO_2 compliance under ethical consideration protocols. The Femur and tibia bones have dissented and muscle tissue wholly removed. Both sides of the bone were cut, and the low-glucose Dulbecco's Modified Eagle's Medium (DMEM) plus 10% fetal bovine serum (FBS), 1% penicillin/streptomycin, nystatin and amphotericin B, and 1% non-essential amino acids (all from Gibco, Carlsbad, CA) were flushed inside the bone to the extraction of stromal bone marrow cells. Finally, the cells were passed through a 70 mm filter (sigma, Z240060, US). The cell viability was determined by trypan blue staining.

In the next step, 25×10^6 cells in a petri dish containing DMEM listed in the above paragraph were placed under standard conditions such as 5% CO_2 at 37°C for 3 hours. Then non-attached cells were removed by exchanging the medium. After 8 hours, the medium was replaced again, and this circle was repeated every 8 hours for 72 hours. The attached cells were washed with phosphate-buffered saline (PBS), and the medium was replaced every three days. After 21 days, the cells' confluency was reached 80-70%. After that, the cells were disaggregated by 25 mM EDTA 1% trypsin for 2 minutes at 37°C. After the neutralization of trypsin, the cells were seeded in 25 cm^2 flasks (26, 27).

Differentiation to osteoblasts and adipocytes. Before treating *S. platensis*, the cells were cultured and osteogenic and adipogenic differentiation media for 14 days (28). These cells were as control and treated differently from *S. platensis* culture. In the second passage, 5×10^4 cell/ml was incubated in a six-well plate with DMEM and 10% FBS to determine the cell potential to differentiate into the bone lineage. The cells were confluence after one week, and the medium was replaced with osteogenic differentiation media. This medium consisted of DMEM, 50 µg/ml ascorbic acid 2-phosphate, 10 nM dexamethasone, and 10 mM β-glycerol phosphate (all obtained from Gibco, United Stat). Plates were incubated at 37°C with 5% CO_2 for 21 days, and the medium was replaced every three days. The cells were fixed with 10% formalin for 10 minutes and stained with Alizarin Red for 20 minutes at room temperature. Finally, they were observed under an optical microscope and took photos (8, 26, 29).

For evaluation of adipocyte differentiation, 5×10^4 cells/ml in the second passage were seeded with DMEM, 50 µg/ml indomethacin, and 100 nM dexamethasone in 6 well plates. The cells were cultured at 37°C with 5% CO_2 for 21 days, and the medium was replaced every three days (30). The cells were stained with 5% Oil Red for 20 minutes to determine adipocyte differentiation and then observed with an optical microscope and took photos (8, 31).

Immunophenotypic analysis. For further characterization, cell surface markers phenotyping was performed for MSCs at second passages. Cell-surface markers were labeled with anti-human antibodies such as CD90 and CD105-fluorescein isothiocyanate (FITC) (Beckman Coulter, Fullerton, CA). Rat isotype antibodies performed as control (Becton Dickinson; Beckman Coulter) (28, 32).

Cell proliferation evaluation. Cell viability was investigated by mitochondrial reactive, named 3- (4, 5-dimethylthiazol-2-yl) -2, 5-diphenyl tetrazolium bromide (MTT assay). The mechanism of this method is the ability to live cells to convert tetrazolium to

formazan (33). The MTT assay was performed by a procedure described in the previous study with slight modifications (25). Briefly, 1×10^4 cells were treated in 96 wells microplate selected with a different logarithmic concentration of *S. platensis* (0, 0.1, 0.3, 0.9, 3, 9, and 30 µl of *S. platensis* per 1 ml DMEM) under standard conditions for 14 days. In parallel, DMEM *spirulina*-free medium was applied as the control group. The medium was replaced every three days. After a predetermined incubation time, the medium was replaced with 15% MTT solution (Atocel, Austria) on PBS (5mg /ml PBS) and then was incubated at 37°C for 2 hours. The wells solution was replaced by Dimethyl sulfoxide (DMSO) (cell-culture grade, Sigma, D2650, US) solution inside a dark chamber and was shaken for 15 minutes. The plate reader measured the optical density (OD) at 570 nm wavelength and 640 nm as reference. The standard curve calculated the number of viable cells. The data obtained from each sample were normalized with those in the negative control, where the control sample's cell viability was considered 100%.

ATP assay. For this assay, rBMSCs at the density of 5×10^4 cells were seeded in 96 wells microplate, and then different concentrations of algae extract were added. After 24 hours, based on the manufacture's protocol, ATP concentration was calculated by Cell Titer Glo® Luminescence assay. The medium of each well was replaced by a mixed solution of 100 µl of fresh culture medium and 100 µl of Cell Titer Glo. The cells were placed on an orbital shaker for 10 minutes to lyse. ATP concentrations were calculated using a standard curve of various concentrations of ATP solutions (34).

Statistical analysis. The growth care was drawn, and then OD obtained from the MTT assay was converted to the number of viable cells. The data were expressed as means ± SD and statistically analyzed by the ANOVA comparison method. A P-value <0.05 was considered as the level of significance.

RESULTS

The differentiation potential of isolated MSCs to osteogenic and adipogenic lineages. The cells isolated from rat bone marrow in passage two are shown in Fig. 1A. Also, Alizarin Red S and Oil Red O staining evaluated the differentiation potential of cells after treating with osteogenic and adipogenic *Spirulina*-free defined media. Calcium deposition is a parameter for demonstrating cell differentiation to osteocytes (Fig. lB). The picture taken from Oil Red O staining depicted cells in round shape with the adipose- vacuole, differentiated adipocytes (Fig. 1C). This can be as a method to prove that the cells are MSCs in the majority.

MSCs marker approvement. The cells isolated from bone marrow were assessed to have MSCs surface CD markers as CD90 and CD105. These markers are specific for mesenchymal stem cells. The results demonstrate that most cultured cells have express-specific markers on their surface (72.6% for CD90 and 77.1% for CD105) (Fig. 2). High expression of these markers can be approved for being the majority of MSCs in the culture.

Antioxidant activity based on DPPH assays. Since DPPH assay is one of the most effective and widely used methods to evaluate the extract's ability as a hydrogen donor or free radical scavenger, this method was used to assess the antioxidant activity of *spirulina platensis.* Fig. 3 shows a dose-response curve of the antioxidant activity of the extract. As the concentration of the extract increases, the antioxidant activity increases. This amount reaches its highest value at 1000 µg/ml (57% inhibition) for extract. All samples show the DPPH radical scavenging activity. The data were also compared with ascorbic acid as a standard antioxidant. The results showed that the extract has a proton-donating ability and the absorption of free radicals of the extract is less than ascorbic acid. The results of this study are consistent with other studies (35, 36).

Antibacterial properties. The antibacterial activity of *spirulina platensis* was investigated against two species of gram-positive bacteria (*Staphylococcus aureus* and *Bacillus subtilis*) and two species of gram-negative bacteria (*Escherichia coli* and *Salmonella typhimurium*). For this purpose, the different concentration of the extract was provided from 1 to 10 mg/ml. Doxycycline (30 µg/ml) was used as a positive control.

As illustrated in Table 1, *spirulina* extract in all concentrations has shown effective antibacterial activity in a dose-dependent manner. This phenomenon was

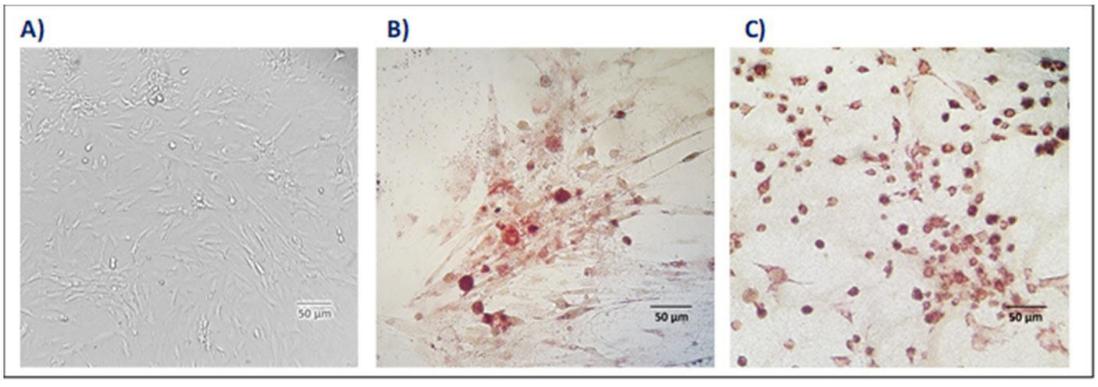

Fig. 1. Microscopic appearance of cells isolated from rat's bone marrow. (A) the primary cells were cultured, and after the second passage, the photo was taken from confluent cells. The cells are spindle and fuzzy in shape and expanded to attach to the surface of the poly styrene culture flask. (B) the cells were differentiated to osteogenic lineage in defined media and then were stained by Alizarin Red. Calcium deposition appears in red on the surface. (C) the isolated cells were differentiated to adipocytes in adipogenic medium and then were stained by Oil Red. The adipocytes appear approximately round in shape with a large vacuole that is visible in red.

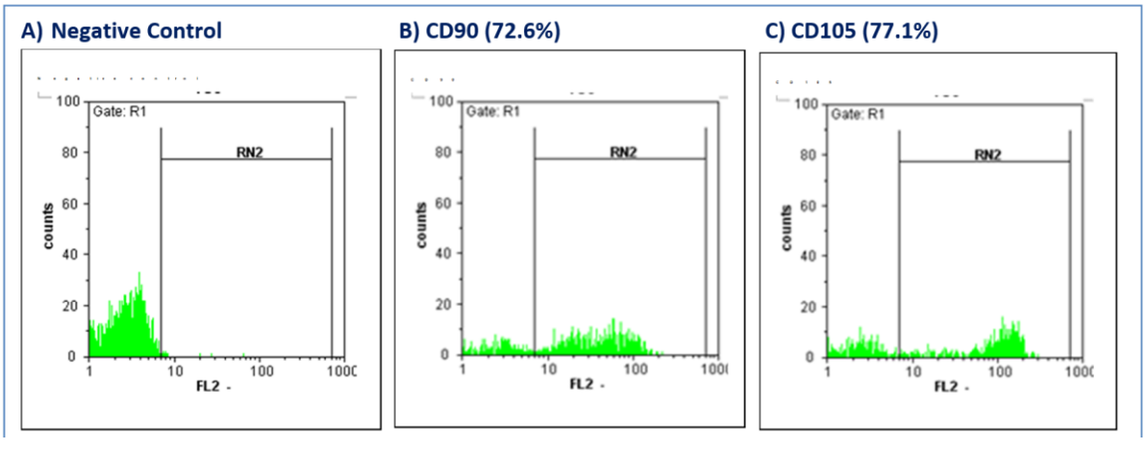

Fig. 2. MSCs derived from rat bone marrow expressed both mesenchymal-specific markers. As illustrated, 72.6% of cells expressed CD90 (B), and 77.1% expressed CD105 (C). Cell-surface markers were labeled with CD90 and CD105-fluorescein isothiocyanate (FITC) anti-human antibodies. Rat isotype antibodies performed as negative control (A).

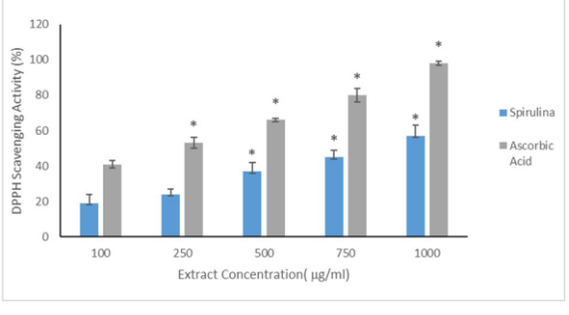

Fig. 3. DPPH-radical scavenging activity of *Spirulina* extract compared with ascorbic acid. Data presented as mean ± standard deviation (n = 3) (* Significant difference, $p < 0.05$). There is an increase in both *Spirulina* and Ascorbic Acid DPPH-scavenging activity in dose-dependent manner.

in agreement with Ozdemir et al. (37). The inhibition zone ranged from 6 to 11 mm, and the most susceptible bacteria were *Escherichia coli* and *Salmonella typhimurium*, with the highest inhibition zone. According to the results, *spirulina* extract has been influential on both types of gram-positive and gram-negative bacteria.

Cell proliferation. The viability of MSCs was evaluated by applying the MTT assay after 14 days of treatment with various concentrations of *S. platensis*. The results (Fig. 4) demonstrated that the first concentration (0.1 µl/ml) increased cell proliferation. The number of viable cells was increased in low concentrations (up to 0.3 µl/ml). In a concentration of 0.9 µl/ml, a sig-

Table 1. Antibacterial activity of *spirulina* extract

Strain. No.	Name of Microorganism	Zone of inhibition at various concentrations of the extract (mm)					Positive control
		1 mg/ml	2 mg/ml	4 mg/ml	8 mg/ml	10 mg/ml	Doxycycline
1	*Bacillus subtilis*	6	7	6	7	7	25
2	*Staphylococcus aureus*	6	7	8	8	9	30
3	*Escherichia coli*	7	9	10	11	10	31
4	*Salmonella typhimurium*	7	7	8	8	7	27

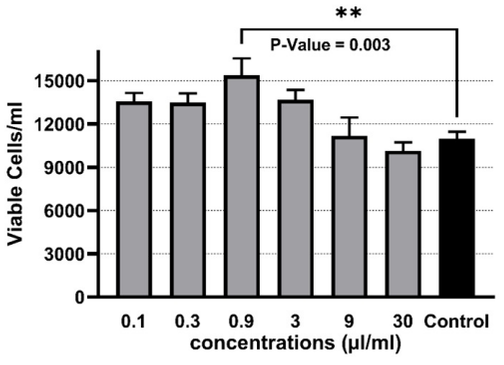

Fig. 4. The MTT assay to evaluate the cell proliferation. 1 × 10^4 cells were cultured for 14 days in logarithmic concentrations (0.1 - 30 µl/ml). The number of cells has increased in range concentration from 1.0 µl/ml to 9 µl/ml. In contrast, in the concentration of 30 µl/ml, the number of viable cells decreased against control. The optimum concentration to proliferation was 0.9 µl/ml. Optical density was measured, and the number of cells was obtained by growth carve normalization. The means and SEM were calculated to ANOVA statistical analysis (the criterion of signification: P-Value < 0.05).

nificant increase can be observed against the control group. In a concentration of 3 µl/ml, the increased behavior of cells was similar to low concentrations (0.1 µl/ml and 0.3 µl/ml). In concentration 9 µl/ml, viable cells are approximately equal to the control group. In contrast to other groups, in concentration 30, the number of cells was dropped. The results illustrated that in concentrations higher than 0.9 µl/ml, the proliferation rate was decreased. In general, although the cells increased slightly in some groups, the optimum concentration to promote MSCs proliferation was 0.9 µl/ml.

ATP assay assessment. To further verification of the MTT assay, MSCs were evaluated by ATP assay in considering proliferation. Microalgae extract in 0.1 µl/ml, and 3 µl/ml concentration did not affect cell viability, meaning they did not alter the number of viable cells during 14 days. The extracts at concentrations of 9 µl/ml caused a slight decrease in cell viability. Concentrations of 30 µl/ml, meanwhile, significantly reduced cell viability by 37%. On the other hand, cell viability increased at a concentration of 0.1 µl/ml, 0.3 µl/ml, and 0.9 µl/ml during the experimental period (Fig. 5).

DISCUSSION

Limited proliferative capacity of bone marrow stromal cells (especially MSCs) can be an important challenge in their applications in cell therapeutics. The proliferation of these primary cells will be decreased after some passages. Depending on environmental conditions, the cells tend to differentiate into various lineages such as osteoblasts, adipocytes, chondrocytes, etc. (38). Some previous studies had been designed to propose methods to promote cell proliferation. For instance, the supernatant of *Lactobacillus acidophilus* has been impressive results on bone marrow stromal cells (8). This study aims to

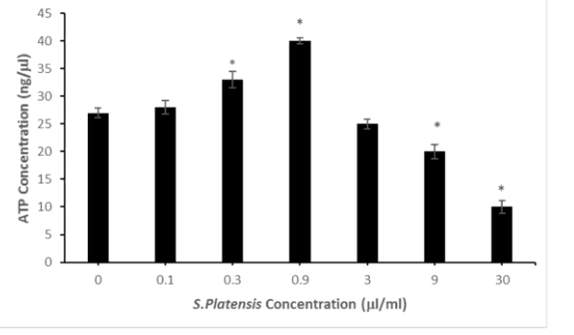

Fig. 5. ATP concentration expressed as mean ± SEM for: control (untreated sample = 0) and MSCs treated with six concentrations of *S. platnsis* (0.1 µl/ml, 0.3 µl/ml, 0.9 µl/ml, 3 µl/ml, 9 µl/ml,30 µl/ml) * P-Value < 0.05 significantly different from the control.

find a method that significantly affects the proliferation of rat bone marrow mesenchymal stem cells. To achieve the Ames of study, *spirulina* micro-algae were used as culture supplements. MSCs were isolated from rat bone marrow and treated with a logarithmic concentration of *S. platensis* for 14 days, according to the previous study (39). Various characteristics of the cells were evaluated before and after treatment to assessments of cell proliferation.

Spirulina contains many biomolecular components, including carotenoids, vitamin E, phycocyanin, and chlorophyll. These compounds have some abilities for hydrogen donating, which reduces the DPPH radicals (40). The results of the present study demonstrated that *spirulina* microalgae have a desirable antioxidant property. This property is dose-dependent and can play an important role in protecting cells against free radicals. Chu Van Lion (2010) revealed that the protective effects of *spirulina* against free radicals are through the inhibition of the cell apoptotic pathways (35). In addition, the results of this study conform to previous studies that imply the mechanism of antioxidants on MSCs by regulation of cyclin-dependent kinase (CDK) and CDK inhibitor levels (41).

According to the published articles, the antibacterial activity of *spirulina* extract is due to its chemical compounds (42, 43). Park et al. showed that γ-linoleic acid in green seaweed *Enteromorpha Linza* induces antibacterial activity (44). It has also been reported that 1-Octadecene, 1-Heptadeceane in some plants and algae have antioxidant, antibacterial, and anticancer activity (45, 46). In this study, it was noticed that increasing the concentration of *spirulina* in the cell culture medium enhances the antibacterial property. Active lipids are found in high concentrations in *Spirulina* (47). Lampe et al. hypothesized that lipids could penetrate the peptidoglycan of the cell wall and reach the bacterial membrane, causing its disintegration (48). Mandit et al. also described the antibacterial mechanism of fatty acids by altering cell membrane permeability, interacting with cell membrane proteins and lipids, and forming a layer around the cell (49). On the other hand, regarding Alshuniaber et al. study (2021), antibacterial activity is also related to the accumulation of phenolic compounds in *Spirulina* (50). At low concentrations, phenols affect the activity of energy production-related enzymes, while at high concentrations, they lead to protein denaturation (51). Therefore, *spirulina* extract is rich in biological compounds with antibacterial properties.

MTT assay was performed to investigate the effects of *S. platensis* on proliferation. As a result, the proliferation of cells had been increased by *S. platensis* treatment, especially in 0.9 µl/ml concentration compared with the control group. Cell proliferation at this concentration was significantly higher than *in vitro* culture in standard condition (DMEM medium and 10% FBS) in previous studies (52). ATP assay indicated that at concentrations of 0.3 µl/ml and 0.9 µl/ml, the level of ATP increased significantly, indicating an enhancement in MSCs' proliferation. It is well known that an increase in the intracellular level of ATP is directly related to an enhancement in the cell's metabolism, leading to increased cell proliferation (53). Both MTT and ATP assays confirm that there are not cell toxicity in extract up to 30 µl/ml on MSCs, and conversely, it can be cause to increase in proliferative activity of these cells. This is despite the fact that in past studies, the proliferation of MSCs has consistently been one of the problems researchers faced, and the proliferation of these cells drops significantly after several passages (54). The *S. platensis* concentrations more than 0.9 µl/ml are a duo to decreasing medium amounts to stabilize cell culture conditions. It can be a limitation to measurements of exact effects of *S. platensis* on MSCs in high concentrations. Therefore, the results achieved from this study depicted that the optimum concentration *in vitro* of *S. platensis* to treat of MSCs is 0.9 µl/ml, and further concentrations of *S. platensis* could not be recommended. These results correspond to some extent to a previous study about *Lactobacillus acidophilus* supernatant published in 2016 (8). However, according to the diagram in Fig. 4, with rising concentrations, the number of living cells declines with a lower slope than predicted. It's probably due to the high levels of nutrients in *S. platensis* extract. Nonetheless, this issue should be studied in detail in the further survey.

Some previous research assessed the effects of *Spirulina* on various cells (35, 55, 56). The release of inflammatory cytokines leads to the production of reactive oxygen species and reduced superoxide dismutase activity. TNF-alpha can be mentioned among these inflammatory factors (57). These processes lead to senescence in cells. Due to the presence of C-phycocyanin in spirulina, the apoptosis would be inhibited by downregulating p53, p21, and p27. Fur-

thermore, spirulina can suppress apoptosis by reducing the expression of Bax. This protein inhibits the mitochondrial pathway of apoptosis, and as a result, the production of cytochrome C will be decreased (58). These studies have emphasized the importance of *Spirulina* as a nutritional element, antioxidant, and antibacterial activity that can be a compound reason for cell proliferation. The increased proliferation of mesenchymal stem cells *in vitro* can lead to improved therapeutic approaches (35). Overall, comparing the effect of some proteins and organic biomolecules secreted from *Spirulina* is recommended for future study. Then, determining the impact of which categories of nutrients in this algae can be expressed as an important issue.

CONCLUSION

The weak proliferative potential of mesenchymal stem cells isolated from bone marrow can be caused some limitations in the cell manipulations. This study confirms that cells treated with various concentrations of *S. platensis* significantly increased the proliferation of MSCs concerning nutrient, antioxidant, and antibacterial activity. The best results will be achieved by purifying secretory proteins from the algae and treating the cells in the optimum concentration.

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REFERENCES

1. Shabnama Kouser A, Arabi MS. Spirulina-an overview. *Int J Pharm Pharm Sci* 2012; 4: 9-15.
2. Campanella L, Russo M, Avino P. Free and total amino acid composition in blue-green algae. *Ann. Chim* 2002; 92: 343-352.
3. Colla L, Bertolin T, Costa J. Fatty acids profile of Spirulina platensis grown under different temperatures and nitrogen concentrations. *Z Naturforsch C J Biosci* 2004; 59: 55-59.
4. Golmakani M, Rezaei K, Mazidi S, Razavi S. γ-Linolenic acid production by Arthrospira platensis using different carbon sources. *Eur J Lipid Sci Technol* 2012; 114: 306-314.
5. Jubie S, Ramesh P,Dhanabal P, Kalirajan R, Muruganantham N, Antony A. Synthesis, antidepressant and antimicrobial activities of some novel stearic acid analogues. *Eur J Med Chem* 2012; 54: 931-935.
6. Babadzhanov A, Abdusamatova N, Yusupova F, Faizullaeva N, Mezhlumyan L, Malikova M. Chemical composition of Spirulina platensis cultivated in Uzbekistan. *Chem Nat Compd* 2004; 40: 276-279.
7. Tokuşoglu Ö, Üunal M. Biomass nutrient profiles of three microalgae: S*pirulina platensis*, Chlorella vulgaris, and Isochrisis galbana. *Curr Res Food Sci* 2003; 68: 1144-1148.
8. Samadikuchaksaraei A, Gholipourmalekabadi M, Saberian M, Alitappeh M, Shahidi Delshad E. How does the supernatant of *Lactobacillus acidophilus* affect the proliferation and differentiation activities of rat bone marrow-derived stromal cells? *Cell Mol Biol (Noisy-le-grand)* 2016; 62: 1-6.
9. Halper J, Leshin L, Lewis S, Li W. Wound healing and angiogenic properties of supernatants from Lactobacillus cultures. *Exp Biol Med (Maywood)* 2003; 228: 1329-1337.
10. Li W, Brackett B, Halper J. Culture supernatant of *Lactobacillus acidophilus* stimulates proliferation of embryonic cells. *Exp Biol Med (Maywood)* 2005; 230: 494-500.
11. Baksh D, Song L, Tuan R. Adult mesenchymal stem cells: characterization, differentiation, and application in cell and gene therapy. *J Cell Mol Med* 2004; 8: 301-316.
12. Eslami-Arshaghi T, Salehi M, Soleimani M, Gholipourmalekabadi M, Mossahebi-Mohammadi M, Ardeshirylajimi A, Rajabi H. Lymphoid lineage differentiation potential of mouse nuclear transfer embryonic stem cells. *Biologicals* 2015; 43: 349-354.
13. Horwitz E, Gordon P, Koo W, Marx J, Neel M, McNall R, Hofmann T, et al. Isolated allogeneic bone marrow-derived mesenchymal cells engraft and stimulate growth in children with osteogenesis imperfecta: Implications for cell therapy of bone. *Proc Natl Acad Sci U S A* 2002; 99: 8932-8937.
14. Bianco P, Riminucci M, Gronthos S, Robey P. Bone marrow stromal stem cells: nature, biology, and potential applications. *Stem Cells* 2001; 19: 180-192.
15. Fuchs E, Segre J. Stem cells: a new lease on life. *Cell* 2000; 100: 143-155.

16. Fierro FA, Kalomoiris S, Sondergaard CS, Nolta JA. Effects on proliferation and differentiation of multipotent bone marrow stromal cells engineered to express growth factors for combined cell and gene therapy. *Stem Cells* 2011; 29: 1727-1737.
17. Chong, SG, Sato S, Kolb M, Gauldie J. Fibrocytes and fibroblasts—where are we now. *Int J Biochem Cell Biol* 2019; 116: 105595.
18. Jahani H, Kaviani S, Hassanpour-Ezatti M, Soleimani M, Kaviani Z, Zonoubi Z. The effect of aligned and random electrospun fibrous scaffolds on rat mesenchymal stem cell proliferation. *Cell J* 2012; 14: 31-38.
19. Minguell JJ, Erices A, Conget P. Mesenchymal stem cells. *Exp Biol Med (Maywood)* 2001; 226: 507-520.
20. Andrade MR, Costa JA. Outdoor and indoor cultivation of *Spirulina platensis* in the extreme south of Brazil. *Z Naturforsch C J Biosci* 2008; 63: 85-90.
21. Kato-Minoura T, Ogiwara Y, Yamano T, Fukuzawa H, Kamiya R. Chlamydomonas reinhardtii tubulin-gene disruptants for efficient isolation of strains bearing tubulin mutations. *PloS One* 2020; 15: e0242694.
22. Barbarino E, Lourenço SO. An evaluation of methods for extraction and quantification of protein from marine macro-and microalgae. *J Appl Phycol* 2005; 17: 447-460.
23. Zhang W-W, Duan X-J, Huang H, Zhang Y, Wang B-G. Evaluation of 28 marine algae from the Qingdao coast for antioxidative capacity and determination of antioxidant efficiency and total phenolic content of fractions and subfractions derived from Symphyocladia latiuscula (Rhodomelaceae). *J Appl Phycol* 2007; 19: 97-108.
24. Kaushik P, Chauhan A. *In vitro* antibacterial activity of laboratory grown culture of *Spirulina platensis. Indian J Microbiol* 2008; 48: 348-352.
25. Saud B, Malla R, Shrestha K. A review on the effect of plant extract on mesenchymal stem cell proliferation and differentiation. *Stem Cells Int* 2019; 2019: 7513404.
26. Sun S, Guo Z, Xiao X, Liu B, Liu X, Tang P-H, Mao N. Isolation of mouse marrow mesenchymal progenitors by a novel and reliable method. *Stem cells* 2003; 21: 527-535.
27. Nadri S, Soleimani M, Hosseni RH, Massumi M, Atashi A, Izadpanah R. An efficient method for isolation of murine bone marrow mesenchymal stem cells. *Int J Dev Biol* 2007; 51: 723-729.
28. Dominici M, Le Blanc K, Mueller I, Slaper-Cortenbach I, Marini F, Krause D, et al. Minimal criteria for defining multipotent mesenchymal stromal cells. The International Society for Cellular Therapy position statement. *Cytotherapy* 2006; 8: 315-317.
29. Bortolotti F, Ukovich L, Razban V, Martinelli V, Ruozi G, Pelos B, et al. In vivo therapeutic potential of mesenchymal stromal cells depends on the source and the isolation procedure. *Stem cell reports* 2015; 4: 332-339.
30. Sekiya I, Larson BL, Vuoristo JT, Cui J-G, Prockop DT. Adipogenic differentiation of human adult stem cells from bone marrow stroma (MSCs). *J Bone Miner Res* 2004; 19: 256-264.
31. Li J, Curley JL, Floyd ZE, Wu X, Halvorsen YD, Gimble JM. Isolation of human adipose-derived stem cells from Lipoaspirates. *Methods Mol Biol* 2018; 1773: 155-165.
32. Kern S, Eichler H, Stoeve J, Klüter H, Bieback K. Comparative analysis of mesenchymal stem cells from bone marrow, umbilical cord blood, or adipose tissue. *Stem cells* 2006; 24: 1294-1301.
33. Aslantürk ÖS. In Vitro Cytotoxicity and Cell Viability Assays: Principles, Advantages, and Disadvantages. Genotoxicity-A Predictable Risk to Our Actual World. 2018.
34. Fuchs C, Schenk MS, Pham L, Cui L, Anderson RR, Tam J. Photobiomodulation response from 660 nm is different and more durable than that from 980 nm. *Lasers Surg Med* 2021; 53: 1279-1293.
35. Chu W, Lim YW, Radhakrishnan AK, Lim PC. Protective effect of aqueous extract from Spirulina platensis against cell death induced by free radicals. *J Ethnopharmacol* 2010; 10: 1-8.
36. Choi Y, Jang J, Koo HJ, Tanaka M, Lee KH, Choi J. Alginate-chitosan Hydrogel patch with beta-glucan nanoemulsion for antibacterial applications. *Biotechnol Bioprocess Eng* 2021; 26: 71-77.
37. Ozdemir G, Karabay NU, Dalay MC, Pazarbasi B. Antibacterial activity of volatile component and various extracts of *Spirulina platensis. Phytother Res* 2004; 18: 754-757.
38. Baksh D, Yao R, Tuan RS. Comparison of proliferative and multilineage differentiation potential of human mesenchymal stem cells derived from umbilical cord and bone marrow. *Stem cells* 2007; 25: 1384-1392.
39. Saberian M, Seyedjafari E, Zargar SJ, Mahdavi FS, Sanaei-rad P. Fabrication and characterization of alginate/chitosan hydrogel combined with honey and aloe vera for wound dressing applications. *J Appl Polym Sci* 2021; 138: 864-873.
40. Gad AS, Khadrawy YA, El-Nekeety AA, Mohamed SR, Hassan NS, Abdel-Wahhab MA. Antioxidant activity and hepatoprotective effects of whey protein and Spirulina in rats. *Nutrition* 2011; 27: 582-589.
41. Zhang J. Peng JP. Enhanced proliferation and differentiation of mesenchymal stem cells by astaxanthin-encapsulated polymeric micelles. *PLoS One* 2019; 14(5):e0216755.
42. Kaushik P, Chauhan M. In vitro antibacterial activity of laboratory grown culture of *Spirulina platensis. Indian J Microbiol* 2008; 48: 348-352.
43. Rao AR, Reddy AH, Aradhya S. Antibacterial properties of *Spirulina platensis, Haematococcus pluvia-*

lis, Botryococcus braunii micro algal extracts. *Curr Trends Biotechnol Pharm* 2010; 43: 809-819.
44. Park NH, Choi JS, Hwang SY, Kim YC, Hong YK, Cho KK, Choi IS. Antimicrobial activities of stearidonic and gamma-linolenic acids from the green seaweed *Enteromorpha linza* against several oral pathogenic bacteria. *Bot Stud* 2013; 54: 39.
45. Mishra PM, Sree A. Chemical investigation of Finlaysonia obovata: part I–a rare triterpene acid showing antibacterial activity against fish pathogens. *Nat Prod Res* 2008; 22: 801-807.
46. Lee YS, Kang MH, Cho SY, Jeong CS. Effects of constituents of Amomum xanthioides on gastritis in rats and on growth of gastric cancer cells. *Arch Pharm Res* 2007; 30: 436-443.
47. Abdel-Moneim A, Saadony ME, Shehata AM, Saad AM, Aldhumri SA, Ouda SM, Mesalam BS. Antioxidant and antimicrobial activities of Spirulina platensis extracts and biogenic selenium nanoparticles against selected pathogenic bacteria and fungi. *Saudi J Biol Sci* 2022; 29: 1197-1209.
48. Amerikova M, Pencheva El-Tibi I, Maslarska V, Bozhanov S, Tachkov KJ. Antimicrobial activity, mechanism of action, and methods for stabilisation of defensins as new therapeutic agents. *Biotechnol Biotechnol Equip* 2019; 33: 671-682.
49. Mundt S, Kreitlow S, Jansen R. Fatty acids with antibacterial activity from the cyanobacterium *Oscillatoria redekei* HUB 051. *E J Appl Psychol* 2003; 15: 263-267.
50. Alshuniaber MA, Krishnamoorthy R, AlQhtani BS. Antimicrobial activity of polyphenolic compounds from *Spirulina* against food-borne bacterial pathogens. *Saudi J Biol Sci* 2021; 28: 459-464.
51. Ambati D, Reddy AH, Aradhya S. Antibacterial properties of *Spirulina platensis, Haematococcus pluvialis, Botryococcus braunii* micro algal extracts. *Curr Trends Biotechnol Pharm* 2010; 4: 809-819.
52. Rojas M, Xu J, Woods CR, Mora AL, Spears W, Roman J, Brigham KL. Bone marrow–derived mesenchymal stem cells in repair of the injured lung. *Am J Respir Cell Mol Biol* 2005; 33: 145-152.
53. Letsiou S, Bakea A, Goff GL, Lopes L, Gardikis K, Weis M, Ouazzani M. Marine fungus Aspergillus chevalieri TM2-S6 extract protects skin fibroblasts from oxidative stress. *Marine Drugs* 2020; 18: 460.
54. Taylor SE, Clegg P. Collection and propagation methods for mesenchymal stromal cells. *Vet Clin North Am Equine Pract* 2011; 27: 263-274.
55. Khan Z, Bhadouria P, Bisen CP. Nutritional and therapeutic potential of *Spirulina*. *Curr Pharm Biotechnol* 2005; 6:373-379.
56. Morist A, Montesinos J, Cusido J, Godia B. Recovery and treatment of *Spirulina platensis* cells cultured in a continuous photobioreactor to be used as food. *Process Biochem* 2001; 37: 535-547.
57. Bachstetter AD, Jernberg J, Schlunk A, Vila JL, Hudson C, Cole MJ, Sanberg C. Spirulina promotes stem cell genesis and protects against LPS induced declines in neural stem cell proliferation. *PLoS One* 2010; 5(5): e10496.
58. Nawrocka D, Kornicka k, Śmieszek A, Marycz K. Spirulina platensis improves mitochondrial function impaired by elevated oxidative stress in adipose-derived mesenchymal stromal cells (ASCs) and intestinal epithelial cells (IECs), and enhances insulin sensitivity in equine metabolic syndrome (EMS) horses. *Mar Drugs* 2017; 15: 237.