

THE EFFECT OF Ag NANOPARTICLES SYNTHESIZED BY THYMUS VULGARIS PLANT EXTRACT ON THE DUNALIELLA SALINA

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This study investigates the effects of varying concentrations of environmentally friendly synthesized silver nanoparticles on the microalga *Dunaliella salina*. Silver nanoparticles were synthesized using thyme (*Thymus vulgaris* L.) extract. The effects of TV-AgNPs on the optical density, cell size, and motility of the microalgae, as well as on biochemical parameters including catalase activity, photosynthetic pigment content, and soluble protein levels, were evaluated. The results demonstrated that even environmentally friendly silver nanoparticles synthesized via the “green” method exerted toxic effects on *Dunaliella salina* cells, leading to reductions in optical density, cell size, and active motility. Biogenic silver nanoparticles decreased the levels of chlorophyll *a* and chlorophyll *b*, while increasing carotenoid content and catalase activity. In addition, exposure to nanoparticles resulted in an increase in soluble protein content. These findings suggest that even biogenic AgNPs induce oxidative stress in *Dunaliella salina* cells. In response to these adverse conditions, the cells activate protective and adaptive mechanisms to enhance survival.

Keywords: AgNPs, oxidative stress, protective mechanisms, *Dunaliella salina*, green synthesis

INTRODUCTION

Modern nanotechnology research is rapidly emerging as a promising field due to its revolutionary and promising results in many areas [1, 2]. Nanoparticles are of great interest because they have unique chemical and physical properties and large surface areas compared to their bulk equivalents. As nanomaterials have become part of our daily lives, exposure of the environment to nanomaterials is inevitable [3].

Despite the numerous scientific studies devoted to the synthesis and investigation of nanomaterials, further research is still required to develop more effective methods for nanoparticle production [4].

According to the literature, compared with many conventional chemicals and pharmaceutical agents, silver nanoparticles (AgNPs) exhibit greater efficacy against antibiotic-resistant bacteria, multidrug-resistant tumor cells, and parasitic diseases, as well as enhanced antioxidant and antifungal properties. Therefore, the synthesis of environmentally friendly and biologically compatible Ag nanoparticles is of considerable importance [5].

It should also be noted that the accumulation of AgNPs in algal cells, the transport of AgNPs throughout the aquatic ecosystem, and the mechanisms of the effects of nanoparticles on living cells are among the important issues to be investigated. Due to the porous structure of the cell wall, AgNPs can be adsorbed on algae or enter the cell [6]. Algae are used in the synthesis of nanoparticles because they have a high capacity for accumulating metals, are easy to cultivate and manage, can grow at low temperatures, and cause less harm to the environment. The most commonly used algae species for the preparation of silver nanoparticles are *Chlorophyceae*, *Phaeophyceae*, *Cyanophyceae*, and *Rhodophyceae* [7].

However, despite the aforementioned findings, only a limited number of studies have investigated the biochemical effects of nanoparticles, including green-synthesized nanoparticles, on living cells, and the available literature often presents contradictory results. Therefore, in the present study, we aimed to investigate the biochemical effects of environmentally friendly synthesized AgNPs on the microalga *Dunaliella salina*.

MATERIALS AND METHODS

The halophilic green microalga *Dunaliella salina* IPAS D-294 isolated from the Masazir salt lake located in the northwest of Baku was used as the object of the study. *Dunaliella salina* cells were cultivated according to the method of Alizadeh, Jalilova, Aliyev [8]. *Dunaliella salina* cells were cultivated in glass photobioreactors at 26°C under an illumination intensity of 4000 lux using a 1.5 M nutrient medium.

Biogenic silver nanoparticles were obtained by the “green synthesis” method. In our study, silver nitrate salt and thyme plant (*Thymus vulgaris* L.) were used to obtain silver nanoparticles.

To prepare the thyme extract, 15 g of thyme plant material was mixed with 250 mL of boiling water and boiled for 5 minutes. The mixture was then cooled and filtered through filter paper, yielding 200 mL of thyme extract. Subsequently, thyme extract was mixed with 0.5 M AgNO₃ solution in ratio 2:1. The mixture was heated, and the synthesis of silver nanoparticles using *Thymus vulgaris* extract (TV-AgNPs) was observed by a visible color change.

TEM analysis revealed that the synthesized TV-AgNPs had a particle size ranging from 4 to 12 nm. Dynamic light scattering (DLS) analysis showed a hydrodynamic diameter of approximately 210 nm, while the zeta potential was measured at -21 mV.

To determine the changes occurring in *Dunaliella salina* cells under the influence of TV-AgNPs, a Carl Zeiss Axio For Scope A1 light microscope and a photoelectrocolorimeter (KFK – 2) were used. The sizes of *D. salina* algae were obtained using the imageJ program on the computer. The optical density of *Dunaliella salina* cells was determined according to the changes in absorbance at 750 nm.

For the purpose of pigment extraction, 5 ml of control and experimental samples were taken and centrifuged at 3000 rpm for 6 minutes. 4 ml of 100% acetone was added to the obtained precipitates.

The concentration of chlorophylls *a* and *b* and carotenoids was determined by spectrophotometric method (Spekol, Germany) and calculated based on Wettstein coefficients using the formulas below:

$$C_a \left(\frac{\text{mg}}{\text{L}} \right) = 9.7841 \cdot D_{662} - 0.990 \cdot D_{644}$$

$$C_b \left(\frac{\text{mg}}{\text{L}} \right) = 21.426 \cdot D_{644} - 4.650 \cdot D_{662}$$

$$C_{a+b} \left(\frac{\text{mg}}{\text{L}} \right) = 5.134 \cdot D_{662} + 20.436 \cdot D_{644}$$

The amount of carotenoids in the total pigment extract is determined using the formula given below:

$$C_{kar} \left(\frac{\text{mg}}{\text{L}} \right) = 4,695 \cdot D_{440,5} - 0,268 \cdot (C_{a+b} \frac{\text{mg}}{\text{L}})$$

The activity of the catalase enzyme was determined by the gasometric method using a device designed for catalase determination.

The amount of protein was determined using the Bradford assay.

The level of intracellular reactive oxygen species (ROS) was determined using the fluorescent probe DCFH-DA (2',7'-dichlorodihydrofluorescein diacetate) by measuring fluorescence intensity at $\lambda_{ex} = 485 \text{ nm}$ and $\lambda_{em} = 530\text{--}535 \text{ nm}$. Fluorescence intensity was expressed as relative fluorescence units (RFU).

The experiments were conducted in triplicate. Statistical analysis was performed using GraphPad Prism10. Only statistically significant data are presented in this article.

RESULTS AND DISCUSSION

In our study, we investigated the effects of environmentally friendly silver nanoparticles synthesized using thyme (*Thymus vulgaris* L.) extract (TV-AgNPs) on the growth, morphological characteristics, and motility of *Dunaliella salina* cells. The results of studies devoted to the study of the effect of silver nanoparticles on the optical density of microalgae cells during two days of incubation are presented in Table 1.

As shown in Table 1, the optical density of *Dunaliella salina* cells decreased under the influence of silver nanoparticles. Reduced cell growth was observed following exposure to TV-AgNPs. After 1 day of incubation, the optical density (OD) of *Dunaliella salina* cells in the control group was 0,98. Exposure to TV-AgNPs at concentrations of 0,1; 0,5 and 1 mg/mL resulted in decreases in optical density of 5%, 15%, and 42%, respectively, compared with the control.

After 2 days of incubation, the optical density of microalgal cells in the control group reached 1,9. In contrast, treatment with silver nanoparticles at concentrations of 0,1; 0,5 and 1 mg/mL reduced the optical density of *D. salina* cells by approximately 16%, 37%, and 62%, respectively.

Table 1. Effect of different concentrations of TV-AgNPs on the optical density of *Dunaliella salina* cells

Samples	1 st day		2 nd day	
	D ₁	D ₂	D ₁	D ₂
Control (DS)	0,3	0,98 ± 0,03	0,3	1,9 ± 0,08
T ₁ (DS+0,1 mg/mL TV-AgNPs)	0,3	0,93 ± 0,02	0,3	1,6 ± 0,06
T ₂ (DS+0,5 mg/mL TV-AgNPs)	0,3	0,83 ± 0,02	0,3	1,2 ± 0,05
T ₃ (DS+1 mg/mL TV-AgNPs)	0,3	0,57 ± 0,01	0,3	0,72 ± 0,03

In our further studies, we observed changes in the size of *Dunaliella salina* cells under the influence of silver nanoparticles.

Table 2. Effect of different concentrations of TV-AgNPs on the size of *Dunaliella salina* cells

Samples	1 st day		2 nd day	
	Lenght, μm	Width, μm	Lenght, μm	Width, μm
Control (DS)	25 ± 1,0	8,5 ± 0,4	26,5 ± 0,5	10 ± 0,3

T₁ (DS+0,1 mg/mL TV-AgNPs)	23,5 ± 1,0	8 ± 0,4	25 ± 0,6	9 ± 0,4
T₂ (DS+0,5 mg/mL TV-AgNPs)	22,5 ± 0,5	7 ± 0,3	23 ± 1,0	7,5 ± 0,3
T₃ (DS+1 mg/mL TV-AgNPs)	19,5 ± 0,5	5 ± 0,1	20 ± 0,5	5,5 ± 0,2

As shown in Table 2, exposure to silver nanoparticles resulted in a decrease in the size of *Dunaliella salina* cells. After 1 day of incubation, the average cell length of *D. salina* in the control group was 25 µm. Treatment with TV-AgNPs at concentrations of 0,1; 0,5 and 1 mg/mL reduced cell length by 2%, 10%, and 22%, respectively, compared with the control.

The average cell width in the control group after 1 day of incubation was 8,5 µm. Exposure to TV-AgNPs at concentrations of 0,1; 0,5 and 1 mg/mL resulted in decreases in cell width of 6%, 18%, and 41%, respectively, relative to the control group.

After 2 days of incubation, the average cell length of *Dunaliella salina* in the control group was 26,5 µm. In comparison, treatment with TV-AgNPs at concentrations of 0,1; 0,5 and 1 mg/mL reduced cell length by approximately 6%, 13%, and 25%, respectively.

The average cell width after 2 days of incubation was 10 µm in the control group, whereas it decreased to 9 µm, 7,5 µm, and 5,5 µm following treatment with 0,1; 0,5, and 1 mg/mL TV-AgNPs, respectively. Thus, compared with the control, reductions of approximately 10%, 25%, and 45% in cell width were observed under the influence of environmentally friendly synthesized silver nanoparticles.

These results indicate that silver nanoparticles exert a negative effect on the growth and development of *Dunaliella salina* cells. The observed reduction in cell size may serve as a biological indicator of cellular stress and toxicity induced by AgNPs.

The toxic effects of TV-AgNPs were also reflected in the motility of *Dunaliella salina* cells.

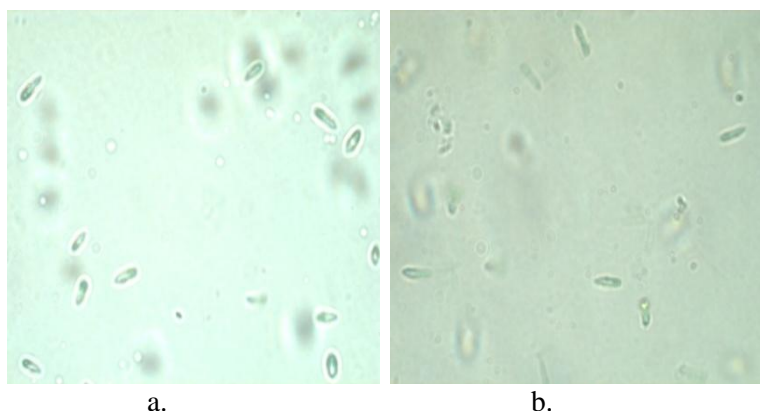


Figure 1. *Dunaliella salina* algae exposed to silver nanoparticles (a – control; b – exposed to 0,1 mg/mL TV-AgNPs)

In the control group, *Dunaliella salina* cells exhibited normal forward motility, whereas significant changes in motility were observed in algae exposed to silver nanoparticles. These observations indicate that exposure to silver nanoparticles impaired the normal motility of the algae (Fig. 1). Specifically, *Dunaliella salina* cells treated with TV-AgNPs were only capable of oscillatory movements in place rather than normal forward movement.

These findings suggest that silver nanoparticles interfere with cellular functions and inhibit the motility of microalgal cells. The loss of forward movement may be associated with disruptions in normal physiological processes and impairment of cellular motility mechanisms. Reduced motility may also limit the ability of microalgae to adapt to environmental conditions. Such effects could result from damage to

the cell membrane and alterations in the mechanical and biological functions of the cells.

Dunaliella salina belong to the phytoplankton species and one of the main physiological processes characteristic of them is photosynthesis. The accumulation of nanoparticles on the surface of algal cells creates a shading effect that affects photosynthesis [9]. This shading effect caused by NPs changes the light, pigments and other conditions essential for photosynthesis, as a result of which the light absorption capacity of algae weakens and the intensity of the photosynthesis process decreases [10, 11].

Chlorophyll a (λ_{\max} ~680 nm) and chlorophyll b (λ_{\max} ~660 nm) are present in many plants and algae, and photons with wavelengths of 660-680 nm provide the highest quantum efficiency. However, the solar spectrum at the Earth's surface has the highest intensity in the blue and green regions of visible light (400-550 nm), and carotenoids have strong absorption in these regions. In photosynthetic organisms, under light, carotenoids accelerate the photosynthesis process by transferring the absorbed excitation energy to chlorophylls, since chlorophylls have weak absorption in this wavelength range. At the same time, carotenoids help protect photosynthetic organisms from the harmful effects of excess light, since they provide triplet-triplet (triplet-triplet) energy transfer from chlorophyll to carotenoid and reduce their harmfulness by attenuating reactive oxygen species (ROS) [12].

β -Carotene is one of the major carotenoid pigments widely distributed in the microalgae *Dunaliella*. This microalgae is able to synthesize β -carotene under extreme environmental conditions such as high light intensity, high salinity, harsh temperature and under conditions of changing pH, or nutrient deficiency [13].

Carotenoids are natural pigments that play important roles in many physiological processes. These pigments have also become popular as food supplements because of their potent antioxidant properties, and may provide protection against diseases such as cardiovascular disease, some types of cancer, age-related macular degeneration, and neurodegenerative diseases such as amyotrophic lateral sclerosis. Recently, microalgae have attracted interest due to their potential to produce novel bioactive metabolites such as carotenoids that provide health and cosmetic benefits [14].

Changes in the amount of photosynthetic pigments directly affect the photosynthetic activity of algal cells [15]. One study showed that nanoparticles alter the rate of photosynthesis and respiration of algae, leading to disruptions in their metabolism [16].

The results of studies devoted to the study of the effect of silver nanoparticles on the amount of pigments in *Dunaliella salina* cells are presented in Table 3.

Table 3. Effect of different concentrations of biogenic Ag nanoparticles on pigment content in *Dunaliella salina* cells

Samples	Pigment content, mg/L					
	1 st day			2 nd day		
	Chl a	Chl b	Caroten.	Chl a	Chl b	Caroten.
Control (DS)	2,67 ± 0,09	1,32 ± 0,03	0,93 ± 0,02	4,86 ± 0,08	2,3 ± 0,06	2,16 ± 0,07
T ₁ (DS+0,1 mg/mL TV-AgNPs)	2,65 ± 0,09	1,33 ± 0,03	0,9 ± 0,02	4,81 ± 0,08	2,3 ± 0,06	2,18 ± 0,07
T ₂ (DS+0,5 mg/mL TV-AgNPs)	2,08 ± 0,08	1,02 ± 0,028	1,39 ± 0,02	4,31 ± 0,07	1,96 ± 0,06	2,4 ± 0,09
T ₃ (DS+1 mg/mL TV-AgNPs)	1,77 ± 0,05	0,78 ± 0,02	1,46 ± 0,03	3,59 ± 0,05	1,5 ± 0,05	2,64 ± 0,1

As can be seen from Table 3, when *D. salina* cells were exposed to TV-Ag-NPs at a concentration of 0,1 mg/L, no significant changes in the content of pigments were observed in both one-day and two-day-old cells. However, changes in the content of pigments were recorded when the concentration of AgNPs in the medium was 0,5 mg/L and 1,0 mg/L.

Thus, after 1 day of incubation, the amount of chlorophyll *a* pigment in *Dunaliella salina* cells

exposed to TV-AgNPs at concentrations of 0,5 mg/mL and 1 mg/mL decreased by approximately 22% and 34%, respectively, and the amount of chlorophyll *b* pigment decreased by approximately 23% and 41%, respectively, compared to the control variant. Regarding the amount of carotenoid pigments, after 1 day of incubation, the amount of carotenoid pigment in *D. salina* cells exposed to TV-AgNPs at concentrations of 0,5 mg/mL and 1,0 mg/mL increased by approximately 49% and 57%, respectively, compared to the control variant (Figure 2.).

After 2 days of incubation, the amount of chlorophyll *a* pigment in *D. salina* microalgae exposed to AgNPs at concentrations of 0,5 mg/mL and 1,0 mg/mL decreased by approximately 11% and 26%, respectively, and the amount of chlorophyll *b* pigment decreased by approximately 15% and 31%, respectively, compared to the control. After 2 days of incubation, the content of carotenoid pigment in *Dunaliella salina* cells exposed to TV-AgNPs at concentrations of 0,5 mg/mL and 1,0 mg/mL increased by approximately 11% and 22%, respectively, compared to the control (Fig.2).

Thus, as can be seen from Figure 2, compared to the control, the content of chlorophyll *a* and chlorophyll *b* pigments in *D. salina* cells incubated with 0,5 mg/mL TV-AgNPs and 1 mg/mL TV-AgNPs decreased, while the content of carotenoids increased. Chlorophylls are the main pigments that play a role in the photosynthesis process by absorbing light, therefore, a decrease in their amount indicates that the efficiency of the photosynthesis process decreases and algae have difficulty absorbing light energy. However, as a defense against these effects, the amount of carotenoids increased. Carotenoids are auxiliary pigments and play an important role in the absorption of light energy in photosynthesis and at the same time these pigments protect algae cells against oxidative stress. The increase in carotenoid content indicates that the cells activate enhanced antioxidant defense mechanisms in response to the toxic effects induced by silver nanoparticles. This response suggests that the algae initiate adaptive and protective processes to neutralize harmful reactive oxygen species generated within the cells. Consequently, such adaptive mechanisms may contribute to increased cellular tolerance and improved resistance to nanoparticle-induced stress.

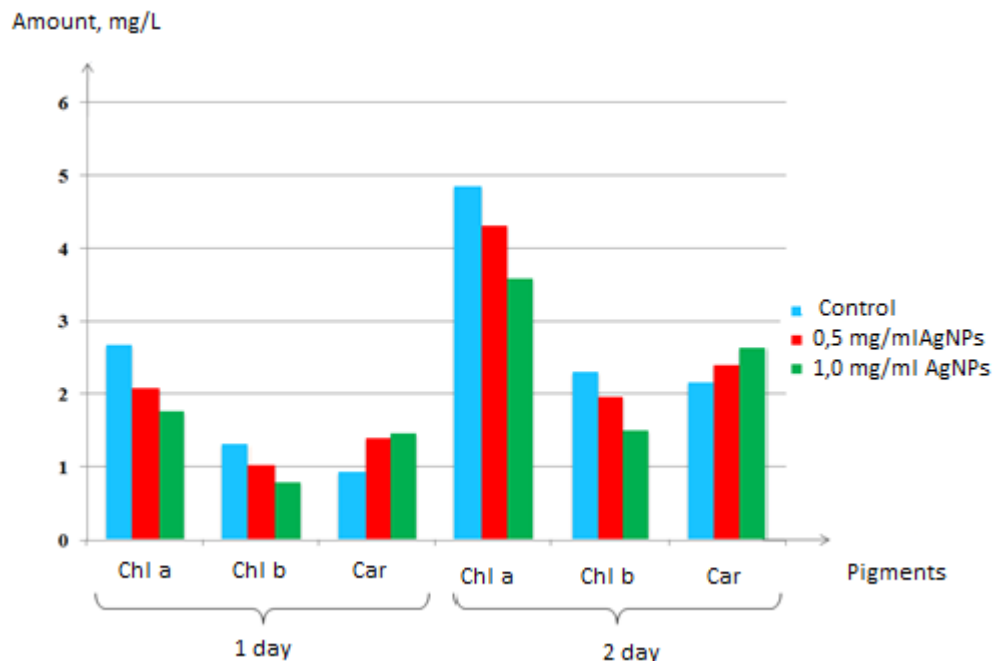


Figure 2. Effects of different concentrations of biogenic AgNPs on photosynthetic pigment content in *Dunaliella salina* cells

Antioxidant defense mechanisms in algae cells include antioxidant enzymes such as superoxide dismutase (SOD), catalase (CAT), glutathione S-transferase (GST) and peroxidase (POD). In the presence of less toxic nanoparticles, the activity of these enzymes increases in order to eliminate the damage of reactive oxygen species to algae cells, but if the toxicity of NPs in the aquatic environment is too high, the antioxidant defense system (ADS) of algae cells is damaged and a significant decrease in enzyme activity occurs [17].

In our research, the activity of the catalase enzyme in *Dunaliella salina* cells treated with biogenic silver nanoparticles was tested at intervals of 5, 10, 15, and 20 minutes by determination of the amount of released oxygen.

As can be seen from Table 4, the amount of released oxygen in *Dunaliella salina* cells in the control variant after 1 day of incubation at the 5th, 10th, 15th and 20th minutes was 0,4 mL O₂/g, 0,83 mL O₂/g, 1,15 mL O₂/g, and 1,43 mL O₂/g, respectively. Compared to the control variant, the amount of released oxygen in microalgae cells after 1 day of incubation increased by approximately 13%, 21%, 20% and 22%, respectively, as a result of the effect of Ag nanoparticles added at a concentration of 0,5 mg/mL. As a result of the effect of Ag nanoparticles added at a concentration of 1 mg/mL, the amount of released oxygen increased by approximately 45%, 33%, 50% and 47%, respectively, after 1 day of incubation.

After 2 days of incubation, the amount of released oxygen in *Dunaliella salina* cells in the control variant at 5th, 10th, 15th and 20th minutes was 0,43 mL O₂/g, 0,78 mL O₂/g, 1,38 mL O₂/g and 2,08 mL O₂/g, respectively. Compared to the control variant, after 2 days of incubation, the amount of released oxygen in algae cells increased by approximately 33%, 58%, 30% and 7%, respectively, as a result of the effect of Ag nanoparticles added at a concentration of 0,5 mg/mL. As a result of the effect of Ag nanoparticles added at a concentration of 1 mg/mL, an increase in the amount of released oxygen in *D. salina* cells after 2 days of incubation was observed by approximately 51%, 83%, 50% and 27%, respectively.

It should be noted that incubation of *D. salina* cells with TV-AgNPs at a concentration of 0,1 mg/mL had practically no effect on the amount of released oxygen.

Based on the amount of oxygen released over specific time intervals, the average rate of the enzymatic reaction was calculated and expressed as the amount of oxygen released per minute per gram of wet weight. In the control group, catalase activity increased by approximately 62% on the second day of incubation. Incubation of algae with silver nanoparticles at concentrations of 0,5 mg/mL and 1,0 mg/mL resulted in increases in catalase activity of 26,5% and 47%, respectively, on the first day of exposure. However, on the second day of incubation, catalase activity in microalgal cells treated with silver nanoparticles was nearly identical to that observed in the control samples.

To confirm the induction of oxidative stress under the influence of nanoparticles, intracellular ROS levels were determined using the DCFH-DA assay. Increased fluorescence intensity was observed in microalgal cells exposed to different concentrations of biogenic silver nanoparticles compared with the control group. Specifically, exposure to AgNPs at a concentration of 0.1 mg/L resulted in an approximately 30% increase in fluorescence intensity, while treatment with 0.5 mg/L AgNPs caused an almost 1.5-fold increase. Exposure to nanoparticles at a concentration of 1.0 mg/L led to an approximately twofold increase in fluorescence intensity relative to the control.

The elevated fluorescence intensity observed in the AgNPs-treated groups indicates enhanced intracellular ROS generation and confirms the development of oxidative stress in the microalgal cells.

Thus, the pronounced increase in catalase activity during the first day may be attributed to the activation of adaptive antioxidant defense mechanisms in response to oxidative stress induced by metal nanoparticles.

Table 4. Effect of different concentrations of biogenic silver nanoparticles on oxygen production by catalase in *Dunaliella salina* cells

Samples	Amount of O ₂ , mLg ⁻¹
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	1 st day				2 nd day			
	5'	10'	15'	20'	5'	10'	15'	20'
Control (DS)	0,4 ± 0,01	0,83 ± 0,02	1,15 ± 0,012	1,43 ± 0,015	0,43 ± 0,01	0,78 ± 0,02	1,38 ± 0,04	2,08 ± 0,05
T₁ (DS+0,1 mg/mL TV-AgNPs)	0,4 ± 0,01	0,81 ± 0,02	1,13 ± 0,012	1,44 ± 0,015	0,42 ± 0,01	0,79 ± 0,02	1,38 ± 0,04	2,1 ± 0,05
T₂ (DS+0,5 mg/mL TV-AgNPs)	0,45 ± 0,01	1,0 ± 0,02	1,38 ± 0,02	1,75 ± 0,03	0,57 ± 0,025	1,23 ± 0,03	1,8 ± 0,04	2,23 ± 0,06
T₃ (DS+1 mg/mL TV-AgNPs)	0,58 ± 0,02	1,1 ± 0,03	1,73 ± 0,04	2,1 ± 0,06	0,65 ± 0,02	1,43 ± 0,04	2,07 ± 0,05	2,65 ± 0,06

According to the literature, nanoparticles also have an effect on proteins [14]. In a study investigating the toxic effects of TiO₂ nanoparticles on *Anabaena variabilis*, exposure of algae to nanoparticles led to an increase in the level of reactive ROS in the cell membrane and the formation of crystals of membrane proteins. At the same time, the cell membrane was also damaged [19].

The effect of biogenic silver nanoparticles on the soluble protein content in *D. salina* cells was investigated, and the results are presented in Table 5.

According to the data presented in Table 5, after 2 days of incubation, the soluble protein content in *Dunaliella salina* cells of sample N1 was 0,3 µg/mL in the control group. At low nanoparticle concentrations, no significant changes in protein content were observed. However, exposure to TV-AgNPs at a concentration of 0,5 mg/mL resulted in more than a twofold increase in protein content, whereas treatment with 1 mg/mL TV-AgNPs caused an approximately threefold increase compared with the control group.

In the 10-fold diluted samples, the protein content in *D. salina* cells after 2 days of incubation was 0,09 µg/mL in the control group. Exposure to TV-AgNPs at concentrations of 0,5 mg/mL and 1 mg/mL increased protein content by approximately 11% and 89%, respectively, relative to the control.

In the 100-fold diluted samples, the protein content in *Dunaliella salina* cells after 2 days of incubation was 0,006 µg/mL in the control group. Treatment with TV-AgNPs at concentrations of 0,5 mg/mL and 1 mg/mL resulted in increases in protein content of approximately 33% and 53%, respectively, compared with the control group.

Table 5. Effect of different concentrations of biogenic silver nanoparticles on protein content in *Dunaliella salina* cells

Samples	Protein content, µg/mL		
	N ₁	N ₂	N ₃
Control (DS)	0,3 ± 0,01	0,09 ± 0,002	0,006 ± 0,0001
T₁ (DS+0,1 mg/mL TV-AgNPs)	0,3 ± 0,01	0,09 ± 0,002	0,006 ± 0,0001
T₂ (DS+0,5 mg/mL TV-AgNPs)	0,7 ± 0,03	0,1 ± 0,0025	0,008 ± 0,0002

T₃ (DS+1 mg/mL TV-AgNPs)	0,9 ± 0,04	0,17 ± 0,003	0,0092 ± 0,0003
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Note: N₁ – sample, N₂ – 10 times diluted sample, N₃ – 100 times diluted sample.

The increase in the amount of proteins in the presence of nanoparticles in the environment indicates that the cell activates its self-protection mechanism and tries to adapt to the changed environment it encounters using protein components.

CONCLUSION

The study demonstrated that even environmentally friendly silver nanoparticles synthesized via the “green” method exerted toxic effects on *Dunaliella salina* cells, leading to reductions in optical density, cell size, and active motility. Biogenic silver nanoparticles decreased the levels of chlorophyll *a* and chlorophyll *b*, while increasing carotenoid content and catalase activity. In addition, exposure to nanoparticles resulted in elevated levels of soluble proteins.

These findings indicate that even biogenic AgNPs induce oxidative stress in *Dunaliella salina* cells. In response to these adverse conditions, the cells activate protective and adaptive defense mechanisms to enhance their survival and resistance to nanoparticle-induced stress.

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