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Comparison of the antibacterial properties of phycocyanin and its SNPs and their effects on rat blood cells and liver enzymes

Mahdieh Izadi[1*] and Elaheh Latifi[2]

Abstract

Background: Phycocyanin is an important protein in cyanobacteria that has many medical and therapeutic properties. The aim of the present study was to compare the antibacterial properties of phycocyanin and its SNPs and to evaluate their effects on rat blood cells and liver enzymes.

Results: The UV absorption in phycocyanin was 620 nm but in phycocyanin nanoparticles was 420 nm. For fluorometry, the maximum emission peak of phycocyanin was 660 nm and that of phycocyanin-AgNO3 nanoparticles was 580 nm. PC-AgNp showed greater antibacterial effects than phycocyanin. In animal studies, it was found that the platelet count in both groups was higher than the control group. Red blood cells and white blood cells had changes. AST and ALT levels increased in both phycocyanin and nanoparticle groups and ALK levels decreased in both groups compared to the control group.

Conclusions: Examination of antibacterial activity showed that PC-AgNp showed more antibacterial effects than PC. Also, in the study of the effect of PC and NP-PC, accumulation of PC and C-Np in mice also altered blood cells and liver enzymes in rats.

Keywords: Antibacterial activity, Blood factors, Liver enzymes, Phycocyanin, Phycocyanin-$AgNO_3$ nanoparticles

1 Background

Phycocyanin is an important protein pigment in cyanobacteria and plays an important role in photosynthesis. Not only is it widely used in food, cosmetics and dyes, but it is also important in applications to improve the body's immunity, promote the regeneration of animal blood cells, fluorescent labeling, etc. [1, 2]. Silver atoms are highly dependent on these functional groups (phycocyanin) and stable nanomaterials can be obtained from them [3, 4]. In addition, C-phycocyanin has some functional groups in proteins, such as amino, carboxyl and mercapto. that can act as a protective agent for nanoparticles for AgNP synthesis. The synthesis of nanoparticles, using metal and metal oxide, through physicochemical and biological methods, is important because of their ability to be used as catalysts and to assist in many processes, including medical physicochemical processes [5]. At present, nanoscience and nanotechnology are rapidly growing and evolving [6]. Synthesis of silver nanoparticles using microorganisms through the recovery of metal ions can be a good alternative to toxic chemicals [7]. Silver nanoparticles are mainly used to combine with plants, fungi and enzymes that have many benefits, such as environmental compatibility and pharmaceutical and biomedical applications, the absence of toxic chemicals [8]. Among the various types of nanoparticles, silver nanoparticles are widely used in biotechnology and medicine [9]. Silver nanoparticles have emerged as a powerful product in the field of nanotechnology and have been widely used in recent years due to their properties such as chemical stability, catalytic activity and antimicrobial properties [10]. Complementary and alternative medicine

*Correspondence: Izadi.mahdieh@yahoo.com
[1] Department of Biochemistry, Payame Noor University, Tehran, Iran
Full list of author information is available at the end of the article

are widely available around the world today. Pigment-producing microorganisms with medicinal properties can be useful for the treatment of various diseases. The pigment phycocyanin in Spirulina Platensis, a high quality protein, has many applications in disease prevention and treatment programs and can be used as a natural and healthy drug without side effects. Also, silver nanoparticles have found wide and increasing applications due to their wide antimicrobial properties. However, nanoparticle toxicity is a topic of discussion, so further studies are needed. In this regard, this study compared the biochemical and antibacterial properties of phycocyanin and its silver nanoparticles and their effect on blood factors and liver enzymes in Wistar rats.

2 Methods

The dried powder of Spirulina platensis was dissolved in distilled water and frozen and thawed for five consecutive cycles. The sample was then incubated in an ultrasonic bath (40 kHz) for 30 min and then centrifuged at 6000 rpm for 30 min and maintained at pH = 7 at 4 °C. For purification, solid ammonium sulfate was gradually added to the crude extract while stirring, reaching 50% saturation. Then, it was stirred for 2 h and stored at 4 °C in the dark for one night. The solution was then centrifuged at 10,000 rpm for 4 min at 4 °C, the supernatant was discarded, and the blue precipitate was dissolved in a small volume of 0.005 M sodium phosphate buffer (pH = 7) and dialyzed against 0.05 M sodium phosphate buffer at pH = 7.

2.1 Biosynthesis of silver nanoparticles

19 ml of phycocyanin extracted from Spirulina platensis was mixed with 1 ml (5 mM) aqueous AgNO3 and incubated for 24 h at room temperature under direct light (2400–2600 lx). Spectroscopies were examined. Their absorption was determined at 620 and 420 nm UV–Vis (SHIMADZU).

2.2 Fourier transformed infrared (FTIR) spectroscopy analysis

The FTIR spectra were used to investigate the functional groups and mechanism of formation of silver nanoparticles, especially to identify the possible interaction between silver precursor salt and protein molecules. PC and NP-PC were blended with KBr and then IR spectral analysis was performed in a Fourier transmission infrared spectrophotometer (JASCO-4200).

2.3 Fluorescence spectroscopy analysis

Fluorescent spectroscopy was used to evaluate the fluorescence properties of phycocyanin as well as its changes after being converted to AgNp PC. The excitation spectra of PC and AgNp PC were measured using a fluorescence spectrophotometer (Scinco, FS-2).

2.4 Comparison of the antibacterial activity of PC and AgNp PC

The antibacterial activity of phycocyanin and its synthesized AgNps was investigated using Gram-positive pathogenic bacteria: *Staphylococcus aureus*) PTCC 1431 (and *Entrecoccus faecalis* (PTCC 1237) and Gram-negative bacteria *Escherichia coli* (PTCC 1338) and *Serratia marcescens* (PTCC 1111) using disk diffusion method. From each of the bacteria, a suspension of 24-h culture was prepared in normal salt, containing 1.5×10^8 CFU/ml of bacteria and in accordance with McFarland's 0.5 standard and cultured in the of Muller Hinton agar medium (Merck- Germany). Cellulose disks (6 mm in diameter) were saturated separately with 20 µl of AgNp PC (5000 mg/ml) and PC (5000 mg/ml). Saturated disk with 5 mm AgNO3 solution, tetracycline and cephalexin disks were used as controls. The plates were incubated at 37 °C for 24 h.

2.5 Animal study

This study was performed on 30 male Wistar rats, about 2–3 months old and weighing 200–250 g, which were purchased from the Royan Research Institute (Isfahan, Iran). The rats were kept at a constant temperature of 25 °C and nutritional status for one week. They were then randomly divided into three groups of 10. The first group (control) received normal water and food. The second group consumed normal food and pure phycocyanin (5000 mg/kg) with water and the third group consumed normal food and AgNp-phycocyanin (5000 mg/kg) with water. After 12 days, blood samples were taken under complete anesthesia of rats so that each rat was placed in a medium containing chloroform impregnated cotton (80 mg/kg), and after complete anesthesia, blood samples were taken and then the rats died.

2.5.1 Hematological examination of rats' blood

The rats' cardiac blood samples were collected to count and examine blood cells in tubes containing EDTA, and after stirring, hematology was performed by Mindray BC-5800 Analyzer Hematology. The parameters analyzed included white blood cell count (WBC), platelets, red blood cell count (RBC), hemoglobin (HGB), hematocrit (HCT), mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH) and mean corpuscular hemoglobin concentration (MCHC).

2.5.2 Biochemical examination of blood of rats

Blood samples were collected in plastic tubes and then centrifuged at a rate of 3000 rpm. Liver enzymes were

tested to evaluate PC and AgNp PC in rat serum by the Mandray BS-200 Biochemistry Analyzer. Liver tests included alkaline phosphatase (ALK), aspartate aminotransferase (AST), and alanine aminotransferase (ALT).

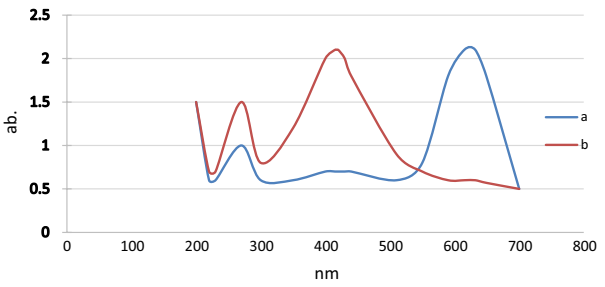

Fig. 1 UV-spectrophotometer diagram, UV absorption diagram, **a** PC, which had the highest UV absorption for PC at 620 nm, **b** AgNPs PC, which had maximum UV absorption for AgNPs PC at 420 nm

2.6 Data analysis

All data were presented as mean ± SEM. Mean values were calculated based on three replicate measurements and all measurements were performed using ANOVA (SPSS 19). The level of significance was set at the $P < 0.05$ level.

3 Results

The biosynthesis of AgNPs PC was characterized by changing the blue to dark brown. After the incubation period, the biodegradation reaction was visualized by visual color change and UV absorption. Specific surface plasmon resonance (SPR) spectra of silver nanoparticles produced by phycocyanin pigment showed an absorption peak at 420 nm, while the highest adsorption of pc was at 620 nm (Fig. 1).

The FTIR spectroscopy results before and after the reaction with silver nitrate are shown in Fig. 2. The FTIR band at 1645 cm^{-1} was assigned to the C=O stretch by N–H deformation, the C=O stretch being assigned to

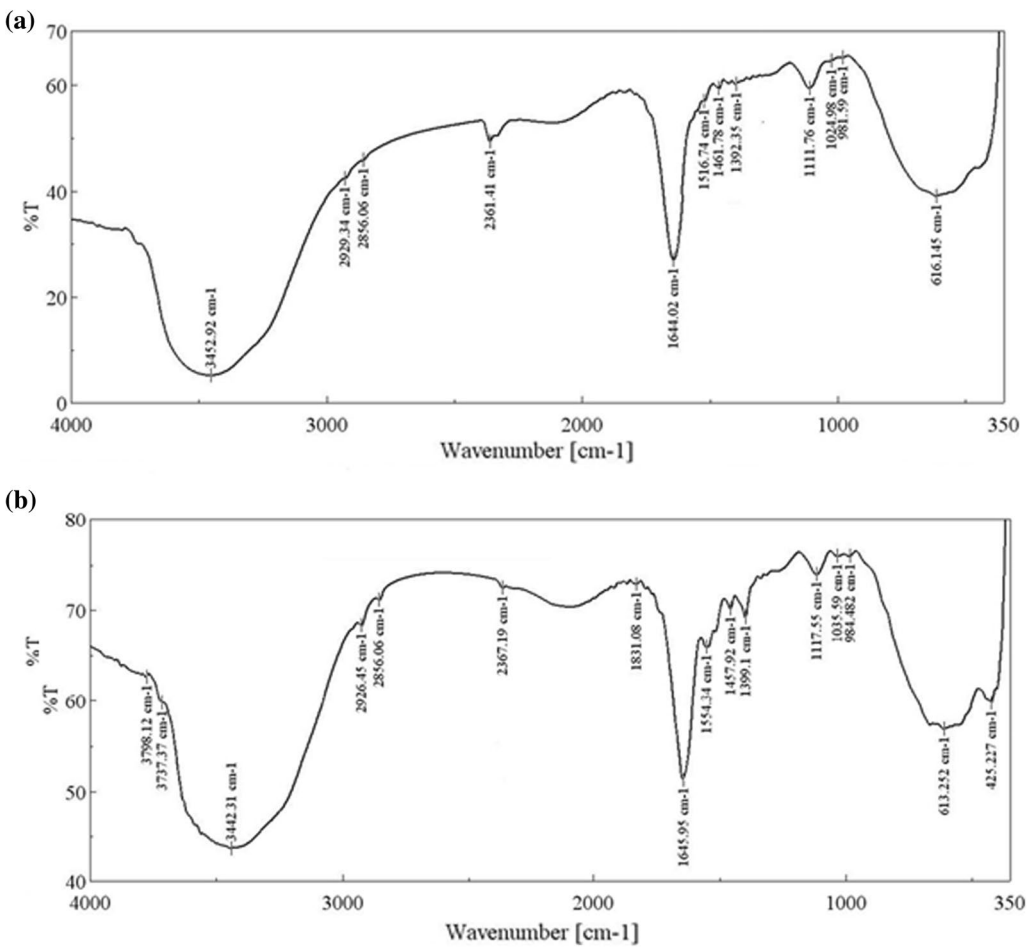

Fig. 2 FTIR spectroscopy, **a** FTIR diagram of PC, **b** FTIR diagram of PC-AgNp

the carbonyl groups [11]. This is a peak in the 984 cm^{-1} amplitude associated with the C–N tensile vibration of the primary amines indicating the possible involvement of the primary amines in the synthesis of the nanoparticles [12].

In the fluorescent study, it was found that the excitation wavelength was 620 nm for PC and 540 nm for PC-AgNp, and the maximum PC emission peaks were 660 nm and PC-AgNp at 580 nm, respectively. Therefore, it was found that PC-AgNp, like PC, has fluorescence properties but differs in excitation and emission wavelengths. Fluorescence spectroscopy diagrams of these two compounds are shown in Fig. 3.

Evaluation of antibacterial activity of PC and PC-AgNp against gram-positive (*Entercoccus faecalis* and *Staphylococcus aureus*) and gram-negative (*Escherichia coli* and *Serratia marcescens*) bacterial species by disk diffusion method in each plate diameter of inhibitory zones around each disks was measured and was found that PC had antibacterial activity only for gram-positive bacteria, but PC-AgNp antibacterial activity was against all four bacteria. The lowest inhibition of phycocyanin was (3 mm) for *Escherichia coli* and the highest was for *Serratia marcescens* (4 mm), the lowest inhibition of PC-AgNp was for *Serratia marcescens* (4 mm) and the highest was for *Entercoccus faecalis* (10 mm) (Table 1).

Hematological examination of rat blood showed that the number of red blood cells decreased in the PC group but slightly increased in the PC-AgNp group compared to the control group. The number of white blood cells increased in the phycocyanin group and decreased in the PC-AgNp group. Hemoglobin levels increased slightly in the phycocyanin group and did not change significantly in the PC-AgNp group. Hematocrit levels were not significantly different in both groups ($p < 0.05$). The platelet count in both groups was higher than the control group (Fig. 4; Table 2).

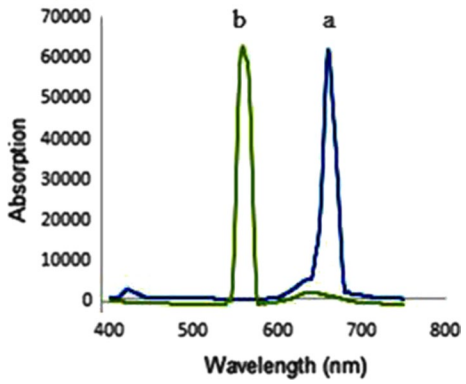

Fig. 3 Fluorescence spectroscopy diagram, **a** PC, **b** PC-AgNp, the excitation wavelength was for PC at 620 nm and for PC-AgNp at 540 nm

Table 1 Evaluation of PC and PC-AgNp antibacterial activity for *Entercoccus faecalis*, *Staphylococcus aureus*, *Escherichia coli* and *Serratia marcescens* based on inhibition zone diameter (mm)

	PC	PC-AgNp	Tetracycline	Cefalexin	$AgNo_3$
	Diameter of inhibition zone (mm)				
Escherichia coli	3	6	9	4	N.I
Entrecoccus faecalis	N.I	10	7	3	N.I
Serratia marcescens	6	4	5	2	N.I
Staphylococcus aureus	N.I	8	10	14	N.I

N.I, no inhibition

Examination of serum levels of rat liver enzymes (AST, ALT and ALK) revealed that AST levels in groups consuming PC and PC-AgNp were significantly increased compared to the control group and ALT levels were increased in both groups. ALK levels were lower in groups consuming PC and PC-AgNp than in the control group and higher in the PC-AgNp consuming group than in the PC group ($p < 0.05$) (Table 3).

4 Discussion

Silver has a wide range of antibacterial and fungicidal activities as well as the ability to coordinate with various ligands and macromolecules in microbial cells and is the noblest metal in the manufacture of nanoparticles. Silver is also widely used in inhibiting microbial proliferation and improving wound healing due to its anti-inflammatory effect. Silver nanoparticles have created new strands in medical protocols, and this has been attributed to their larger volume due to the apparent reaction of silver nanoparticles to the surface [13, 14]. In the present study, a biological method was used to produce silver nanoparticles using phycocyanin extracted from Spirulina platensis. Due to the revival of silver ions and the production of nanoparticles, the color of the samples has changed from blue to dark brown. This discoloration is due to the interaction of phycocyanin and the solution. Silver salt is considered the first indication of the production of silver nanoparticles. The discoloration occurs for the silver nanoparticles due to the oscillation of free electrons in the reaction mixture of the nanoparticles. After 24 h of incubation, the reaction mixture showed that the dark brown dye indicates complete synthesis of the nanoparticles [15]. The peak absorption spectrum of nanoparticles in the spectrophotometer is about 420 nm and confirms the production of silver nanoparticles, which is similar to the results of previous studies [16].

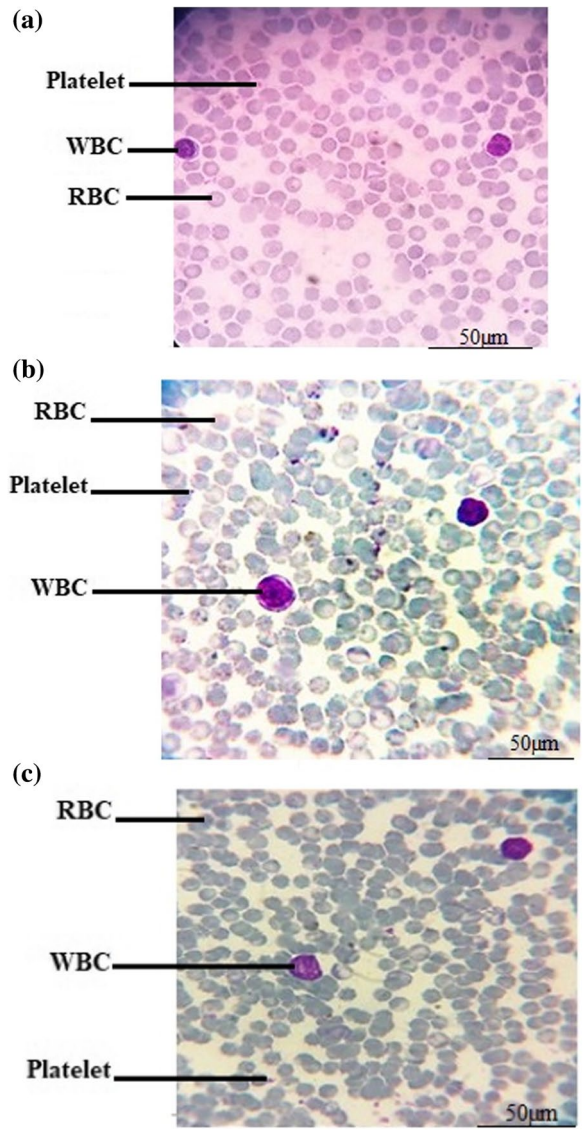

Fig. 4 Microscopic photographs taken from blood parameters of rats, **a** the group taking phycocyanin, **b** the group taking AgNp – phycocyanin, **c** control group

FTIR analysis was performed to investigate the potential organic compounds present in the nanoparticles. After reacting with silver nitrate, some displacements occur at the location and height of the peaks. These displacements are in 3452, 2361, 1152, which are related to the NH, and (H–C=O, C≡N) and C–O tensile strength. Silver salts and precursor proteins reduce silver ions and stabilize silver nanoparticles. The FTIR spectrum supports the presence of a protein on the surface of biosynthesized AgNPs, which causes the metabolic proteins produced to act as coatings during production, reducing the accumulation of silver particles. It has been suggested

Table 2 Comparison of hematological parameters in different groups of rats comparison of hematological parameters in different groups of rats (control, PC consuming group and PC-AgNp consuming group)

Parameter	PC-AgNp	PC	Normal control
RBC (10^{12}/L)	7.7 ± 0.35	7.89 ± 0.20	7.67 ± 0.24
WBC (10^{9} /L)	9.1 ± 0.32	14.7 ± 0.17*	11.2 ± 0.39
HGB (g/dL)	14.9 ± 0.11	15.5 ± 0.17*	14.9 ± 0.23
HCT %	44.3 ± 0.67	44.3 ± 0.78	44.8 ± 0.81
MCV (fL)	57.6 ± 7.5	59.2 ± 0.91	58.5 ± 0.83
MCH (pg)	19.3 ± 0.64	20.6 ± 0.73	19.4 ± 0.67
MCHC (g/dL)	33.6	34.9	33.2
Platelet (10^{9} /L)	112 ± 6.92*	684 ± 23.5	152 ± 7.81

*Values are expressed as mean ± standard error of mean ($p < 0.05$), $n = 5$ rats in each group

Table 3 Comparison of liver enzymes (AST, ALT, ALK) in control, PC consuming group and PC-AgNp consuming groups of rats

Parameter	PC-AgNp	PC	Normal control
AST (U/L)	226 ± 5.1*	285 ± 6.9*	152 ± 5.1
ALT(U/L)	276 ± 12*	225 ± 4.9*	165 ± 0.4
ALK(U/L)	1023 ± 205*	1182 ± 115*	1511 ± 325

AST, aspartate amino transfrase; ALT, alanin amino transfrase; ALK, alkalin phospatase

*Values are expressed as mean ± standard error of mean ($p < 0.05$), $n = 5$ rats in each group

that the stability of AgNPs may be due to the presence of a protein cap agent that encloses them and forms a layer that protects the nanoparticles from aggregation [16–19]. The excitation wavelength of PC-AgNPs was 580 nm and the maximum emission peak was 625 nm. In another research, fluorescence spectrum analysis of the synthesized DNA-Ag nanoparticles showed that the maximum excitation wavelength was at 560 nm and the emission wavelength was 650 nm [20]. A similar study showed that nanosilver synthesized with short hydrogen-based peptides has an excitation wavelength at 530 nm and a diffusion wavelength of 634 nm [21].

In the present study, the antibacterial effect of synthesized silver nanoparticles on standard strains (*Entercoccus faecalis*, *Escherichia coli*, *Serratia marceseus* and *Staphylococcus aureus*) was investigated.

Biosynthesized AgNPs significantly inhibit the growth of Gram-positive and Gram- negative bacteria pharmacologically. The antibacterial activity of silver nanoparticles by breaking the plasma membrane or blocking the respiration associated with oxygen and sulfhydryl groups in the cell wall leads to bacterial cell death. Phycocyanin pigments were evaluated against bacterial species which

in the case of the antibacterial activity against Staphylococcus aureus, this result is almost consistent with previous studies. The release of Ag may inactivate the production of certain enzymes and cellular proteins necessary for the synthesis of adenosine triphosphate (ATP) or the replication of bacterial DNA. Other research has suggested that silver ions may impair the function of restricted membrane enzymes in the respiratory chain. The antibacterial activity of silver nanoparticles is affected by particle size, and the smaller the particle size, the greater the antibacterial effect [22, 23].

Toxicological studies have shown that consumption of medicinal plants or medicines can alter the level of normal hematology [24]. Thus, hematological parameters can be a tool for investigating the effects of drugs [25]. In this study, RBC parameters such as Hb, MCV, MCH and MCHC in rats were studied. It was found that phycocyanin had an additive effect on RBC level and improvement of its parameters. It can be concluded that it can be effective in the treatment of anemia. PC-AgNp had less effect on these parameters. White blood cells (WBCs) kill foreign substances. A number of WBCs have been known to enhance the immune mechanism against toxins [26]. Leukocytes have been reported to be activated by AGEs (Advance Glycated End products), oxidative stress, angiotensin II, and anti-inflammatory cytokines [27]. In this study, rats treated with PC showed more leukocytosis than control and PC-AgNp treated rats.

The number of platelets is directly related to the number of WBCs, indicating a common mechanism. Increased platelet counts are commonly seen in infectious diseases and inflammatory reactions [28]. In the present study, there was an increase in platelet count in phicocyanin-treated rats and a decrease in platelet counts in phycocyanin nanoparticles-treated rats compared to control rats. Therefore, nanoparticle particles influence the coagulation process, which depends on the size, charge, shape and composition of Np [29].

Liu et al. found that intravenous injection of hydroxyapatite nanoparticles increased AST, ALT, and ALP in rabbits [30]. However, intraperitoneal injection does not alter these factors in rat serum, but induces apoptosis in liver and kidney cells [31]. In a similar study, Susan et al. reported that the different effects of nanoparticles are directly related to their diameter and dispersion in body tissues [32]. In fact, free radicals of silver nanoparticles attack liver cells and release their stored ATP into the bloodstream, and through the immune response to an exogenous factor in mice, the number of white blood cells increases and silver nanoparticles are destroyed. [33]. In evaluating the effect of silver nanoparticles in wistar rats, it was found that PC accumulates mainly in the liver, but in oral Np consumption, the levels of AST, ALT and Alp were different and showed partial inactivation of these enzymes [34]. In the present study, both ALT and AST levels were increased in the oral PC and PC-Np injection, but the ALK level was decreased, which was relatively high in the PC-Np group. In separate studies, the potential of silver nanoparticles to modulate enzyme activity depended on thiols [35, 36].

5 Conclusions

Phycocyanin was extracted from Spirulina platensis and its nanoparticles prepared by AgNo3. UV–vis, FTIR and fluorescent spectroscopy confirmed the creation of PC-AgNp. UV absorption was at 620 nm for PC, but was at 420 nm for PC-AgNp. For fluorometry, the peak largest of PC emission was the 660 nm and PC-AgNp was 580 nm. The antibacterial activity of PC and NP-PC for gram-positive bacteria (*Entercoccus faecalis and Staphylococcus aureus*) and gram-negative bacteria (*Escherichia coli* and *Serratia marcescens*) were compared by disk diffusion method, and it was found that PC-AgNp showed more antibacterial effects than a PC. They were also orally administered to rats to investigate the effect of PC and PC-Np on blood factors and liver enzymes of the rat. The accumulation of PC and PC-Np in the rat body caused changes in blood cells and liver enzymes in rats. Platelet count was higher in both groups than the control group. Erythrocytes and white blood cells had changes. AST and ALT levels increased in both groups of phycocyanin and nanoparticles. ALK levels decreased in both groups compared to the control group.

Abbreviations

SNPs: Silver nanoparticles; PC: Phycocyanin; PC-AgNp: Phycocyanin-$AgNO_3$ nanoparticles; PC-Np: Phycocyanin nanoparticles; UV: Ultraviolet; FTIR: Fourier transfer infrared; AST: Aspartate aminotransferase; ALT: Alanine aminotransferase; ALK: Alkaline phosphatase; WBC: White blood cell; RBC: Red blood cell; HGB, Hb: Hemoglobin; MCV: Mean corpuscular volume; MCH: Mean corpuscular hemoglobin; MCHC: Mean corpuscular hemoglobin concentration; HCT: Hematocrit.

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Author contributions

EL contributed to conception and design of study. MI contributed to acquisition of data analysis and/or interpretation of data. EL contributed to drafting the manuscript. MI contributed to revising the manuscript critically for important intellectual content. MI and EL contributed to approval of the version of the manuscript to be published (the names of all authors must be listed). All authors have read and approved the manuscript.

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Availability of data and materials

Not applicable.

Declarations

Ethics approval and consent to participate
The care of animals was carried out in accordance with the guidelines of the Council of European Communities of 24 November 1986 (EEC/609/869) and the guidelines of the National Institutes of Health for the care and use of laboratory animals (NIH Publication No. 8023, amended in 1978).

Consent for publication
Not applicable.

Competing interests
The authors declare no competing interests.

Author details
[1]Department of Biochemistry, Payame Noor University, Tehran, Iran. [2]Department of Biology, Payame Noor University, Tehran, Iran.

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