Expression of Lasr and Lasi Genes in Quorum Sensing System Under the Influence of Iron Oxide Nanoparticles Modified with Silver Surface Structure in the Clinical Strain of Pseudomonas Aeruginosa

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ABSTRACT: *This study was an attempt to examine expression of LasR and LasI genes in quorum sensing system under the influence of iron oxide nanoparticles modified with silver surface structure in the clinical strain of Pseudomonas aeruginosa. The participants of this study 51 samples of Pseudomonas aeruginosa bacteria (samples from different sources) such as Wounds, blood, urine and sputum (from Milad Hospital) in January 2019 to May 2020. The steady development of antibiotic resistance in various bacteria poses a high-risk threat to the health of the global community. Activation of quorum sensing signaling and biofilm formation leads to antimicrobial resistance of pathogens, thus increasing the difficulty in treating bacterial diseases. In this study, 50 clinical samples of patients were prepared in Milad Hospital, Tehran. By confirmatory and differential tests, these samples were confirmed as strains of Pseudomonas aeruginosa. After obtaining the MIC of iron oxide nanoparticles, this concentration (MIC = 0.512) of the nanoparticles was applied to the bacteria. The effect of iron-oxide surface nanoparticles on bacterial pathogens and genes affecting it (LasI and LasR) was investigated. Therefore, in this study, the expression of LasR and LasI genes in quorum sensing system under the influence of iron oxide nanoparticles modified surface structure with silver was investigated in the clinical strain of Pseudomonas aeruginosa.The analysis of data revealed that In microbiological (phenotypic) studies, the effect of iron oxide nanoparticles on surface structure modified with silver led to the lack of growth of Pseudomonas aeruginosa. The expression of LasR and LasI genes was significantly reduced under the influence of iron oxide nanoparticles modified surface structure with silver.In this study, the fidings that iron oxide nanoparticles modified by surface structure can be effective on preventing pathogens by Pseudomonas aeruginosa. Also, surface iron oxide modified silver nanoparticles have antimicrobial properties in inhibiting bacterial growth there are various that are applied through different mechanisms. Decreased expression of LasR and LasI genes causes our nanoparticles to inhibit the quorum sensing system. Therefore, this study proposes to investigate the synergistic effect of iron oxide nanoparticles with antibiotics and other genes from the quorum sensing system.*

KEYWORDS: Pseudomonas aeruginosa, Quorum Sensing, LasI gene, LasR gene, Biofilm, Iron oxide nanoparticles Modified surface structure with silver

INTRODUCTION

Beginning of the 21st century, overuse and indirect use of antibiotics has led to the production of multidrug-resistant bacterial strains (MDRs). Today, entering the post-antibiotic era, global concern is diminishing our ability to fight germs. Pseudomonas aeruginosa causes infections of the urinary tract, respiratory system, inflammation and inflammation of the skin, soft tissue infections, bacteremia (presence of bacteria in the blood), bone and joint infections, gastrointestinal infections and various systemic infections, especially in patients with severe burns. AIDS, whose immune systems are suppressed.

Quorum sensing (QS) is a cell-to-cell communication in which specific signals are activated to coordinate pathogenic behaviors and help bacteria adapt to defects. Activation of QS signaling and biofilm formation leads to antimicrobial resistance of pathogens, thus increasing the difficulty in treating bacterial diseases. Anti-QS agents can suppress QS signaling and prevent biofilm formation, thus reducing bacterial pathogenicity without resistance to anti-pathogen drugs. Anti-QS agents are potential alternatives to antibiotics. This study focuses on anti-QS agents and their mediated signals in pathogens and the potential for targeted QS treatment for bacterial diseases. Iron oxide nanoparticles have attracted much attention due to their extraordinary paramagnetic properties and potential biomedical applications due to their biocompatibility and non-toxicity.

Years before Pseudomonas aeruginosa was identified, doctors at the time considered their observations of blue-green pus to be an important sign that the infection was serious. In 1850, sedillot first noticed the presence of blue-green spots on the clothes of the displaced. In 1860, fordos succeeded in extracting this pigment from the bacteria and named the crystalline substance pyocyanin. In 1862, Luke reported these colored spots in connection with infections and stated that he had observed rod-shaped elements in this blue-green pus. Gessard (1882) isolated the bacterium Pseudomonas aeruginosa and named it Bacillus pyocyanus. The migula (1894) announced the initial characteristics of Pseudomonas aeruginosa.Wasserman (1896) stated that the role of Pseudomonas aeruginosa toxins and extracellular substances was more important than the bacterial cell itself in its pathogenicity. Osler (1952) stated that Pseudomonas aeruginosa may be involved in secondary infections (1-2).

Sangiliyandi et al (2014) investigated the effect of silver nanoparticles on the bacterial biofilm of a number of pathogenic bacteria. The results showed that silver nanoparticles inhibited the biofilm of Pseudomonas aeruginosa, Shigella flexneri, Staphylococcus aureus and Streptococcus pneumoniae. They also showed that silver nanoparticles potentiate the lethal effect (synergistic effect) of ampicillin and vancomycin antibiotics against gram-negative and gram-positive bacteria (3). Amini Bazanjani et al (2016) Studied and identified chromosome genes in Pseudomonas aeruginosa strains isolated from human clinical specimens by multiplex PCR and antibiotic resistance. LasI, LasR and rhIR genes were present in 60%, 48% and 0.5 samples, respectively. However, rhlI and LasB genes were not observed in any of the samples (4). Mollai et al (2013) examined the specific primers of LasI gene for rapid detection of Pseudomonas aeruginosa. In this

46

study, out of 40 isolated clinical samples, 48% had LasI gene (5). Girard et al. (2008) stated that the lasI and lasR genes of the querum sensing (QS) genes are essential for infection and that the QS gene is unique and protected by each bacterium (6). Fuqua et al. (2002) stated that Pseudomonas aeruginosa has the ability to adapt to its environment by regulating the extensive and complex transcription of a repository of pathogenic genes. A key player in this response is the Chromium Sensing (QS) cellular communication system, which coordinates the behavior of Pseudomonas aeruginosa communities. In fact, the transcriptional regulation of many pathogenic genes is controlled by two N-acyl homoserine (AHL) -dependent QS systems called LasI R and Rhl R (7). Pham et al (2019). investigated the inhibitory effect of FeooH synthetic iron oxide nanoparticles on the inhibition of biofilm formation in Pseudomonas aeruginosa. Increasing the concentration of FeooH increased the inhibition of biofilm formation. Microscopic observations confirmed the disturbance of biofilm architecture in the presence of FeooH. (8). Khalid et al. (2019): reported an easy method for the synthesis of silver nanoparticles coated with rhmnolipid and iron oxide and their effect on the biofilms of Staphylococcus aureus and Pseudomonas aeruginosa. RL-coated nanoparticles showed very high anti-adhesion and anti-biofilm activity against Pseudomonas aeruginosa (88%) and Staphylococcus aureus (91%). (9).

Sathyanarayanan et al (2013) investigated the effect of gold oxide and iron oxide nanoparticles on the biofilms of Staphylococcus aureus and Pseudomonas aeruginosa. In this study, it was reported that biofilm growth decreased at higher concentrations of gold and iron oxide nanoparticles compared to the absence of nanoparticles. Therefore, nanoparticles with appropriate concentrations can show a significant reduction in biofilm formation. (10).

Lee et al. (2014) studied the effect of ZnO nanoparticles on pathogens in Pseudomonas aeruginosa and concluded that zinc ions and ZnO significantly inhibited biofilm formation and pyocyanin production in Pseudomonas aeruginosa. (11). Zhang et al (2021) examined the effect of silver nanoparticles coated with polylysine and polyethyleneimine for the treatment of Pseudomonas aeruginosa in vitro and concluded that Ag nanoparticles effectively prevent biofilm formation and multidrug resistance in Pseudomonas Inhibits aerogenesis and also promotes the expression of genes associated with oxidative stress and increases the amount of reactive oxygen species.(12). Akther et al (2020) studied the biosynthesis of silver nanoparticles by fungal cell filtration and sensing their anti-quorum sensing factors against Pseudomonas aeruginosa, and found that macrosilver nanoparticles inhibited the production of pyocyanin, extracellular polysaccharide, and biosynthesis of Pseudomonas aeruginosa. (13). Therefore, the aim of this study was to investigate the effect of silver nanoparticles structured with silver in the clinical strains of Pseudomonas aeruginosa on the expression of las I and las R genes in the quorum sensing system.

METHODOLOGY

Participants
Data was collected from 51 samples of Pseudomonas aeruginosa bacteria (samples from different sources) such as Wounds, blood, urine and sputum (from Milad Hospital) in January 2019 to May 2020.Differential diagnostic tests were performed to confirm them. Bacterial isolates on the

environmentBlood agar culture was transferred to the laboratory. In this study, nanoparticles were used to investigate the effect of nanoparticles of Iron oxide (Fe2O3) surface structure with silver on the expression of LasI and LasR genes effective in formation Pseudomonas aeruginosa biofilm purchased from Dipetronic Laboratory Equipment Company.

Instuments
51 strains of Pseudomonas aeruginosa using biochemical tests such as hot staining, growth in McKangie Agar medium, reaction in TSI medium, of test, mobility check, indole and gas production, growth inv 42 ° C and production of pyocyanin pigment in Müller Hinton agar medium, urease tests, Oxidase and catalase were confirmed. These strains underwent structural modification under the influence of iron oxide nanoparticles Surface with silver on the growth of Pseudomonas aeruginosa at different concentrations were examined.

Preparation of microbial suspension equivalent to .5 McFarland
To prepare a microbial suspension equivalent to half a McFarland of fresh colonies (18 hours until 24) and pure -Pseudomonas aeruginosa inoculated into a tube containing sterile saline, then compared Turbidity with Half McFarland Standard Solution Turbidity, Microbial Equivalent Microbial Suspension was prepared. Optical absorption or OD should be. /1 to. / 8 in the wavelength of 625 nanometer. The amount of turbidity in these methods is very important, so in choosing the sample amount should be careful .No more or less than half a McFarland sample.

Concentration effect - MIC of modified iron oxide nanoparticles structured with silver on the strains
At this stage, iron oxide nanoparticles structured with silver according to the following steps have an effect on bacterial strainswere given:
1 - 1 McFarland bacteria in culture medium. / 5 TSB solution
2 - Preparation of nanoparticles with a concentration of 4 mg / ml, for the effect of nanoparticles with a concentration of MIC = 1 mg / ml on the strains
3- 75 μl of TSB medium was added to each of the 96 well plates.
4- Adding 75 μl suspention of. /5 Macfarland in every well plate except controlwell.
5- 75 μl nanoparticles with a concentration of 1 mg / ml were added to the wells except the control wells. For each of the 51 bacterial strains, 3 replications were considered. Incubate after 24 hours The microplates were taken from 3 wells corresponding to each nanoparticle concentration of 111 μl and placed in vials.RNase free entered. Keep recording the contents of each vial on them and do it. The RNA extraction step was stored in a 71 ° C freezer.

RNA extraction to study lasI gene expression
RNA extraction was performed using the kit of Yekta Tajhiz Azma Company with NO: YT9166 cat.
The procedure is as follows:
First, the bacterial suspension was centrifuged at 911 g for 2 minutes to obtain bacterial precipitate. Remove the culture medium on the sediment, and then add 1 ml of RL solution, then vortex
To lysis the cells.

211 µl of chloroform was added to the vial and then vortexed for 15 seconds.

The vial was centrifuged at 911 g for 11 15 minutes. At this stage, inside the vial, there are 3 layers on top,

Middle and lower are formed, which include RNA solution, protein precipitate and chloroform, respectively.

In this part, the phase on the vial was transferred to a new RNase vial.

The samples were transferred to a column and then centrifuged at 911 g for 1 minute (solution below)

Column removed (.

Add to wash column 511 µl of wash buffer 1 then centrifuge for 1 minute at 911 g

The solution under the column was removed.

Add to wash column 511 µl of wash buffer 2 then centrifuge for 1 minute at 911 g

The solution under the column was removed.

Add to wash column 511 µl of wash buffer 2 then centrifuge for 1 minute at 911 g

The solution under the column was removed (this step is a repetition of step 9).

The column was centrifuged for 2 minutes then transferred to a new RNase Free vial.

RNase Free was added to column 31 111 µl of water. Then incubate after 51 minutes

The vial was centrifuged at 411 g for 1 minute.

Finally, the obtained RNA was stored at 71 ° C.

Qualification - RNA extracted

At this stage, the amount and purity of the extracted RNA was determined using a nanodrop device.

Absorption ratio

Optical RNA sensing at 261 nm to 281 nm and 261 nm to 231 nm

The light absorption ratio was 260/280 nm between 1/8- 2/2. From examples that have a light absorption ratio of 260/230 was more than 2.2 and used for cDNA synthesis.

Preparation - cDNA extracted from RNA

To make a complementary cDNA strand from RNA extracted from a unique kit equipped with Cat No: YT611-based on company instructions was used.

First, the reaction mixture was prepared according to Table 2 and 3 in a sterile nucleotide free of enzyme nuclease-1:

CDNA Synthesis reaction compounds

Template RNA	Total RNA 1000ng	10 µL
Primer	Random hexamer primer (50µM)	1.0µL
Depc- treated water	-	2.4µL
Total Volume	-	13.4

After centrifugation to bind the primer, mix at 71 ° C for 5 minutes

The incubation mixture was then incubated to prevent further unnecessary reactions was placed.

To prepare a cDNA synthesis reaction mixture from RNA, the DNA synthesis reaction compounds of following Tables It was mixed and centrifuged after mixing.

Statistical analysis of gene expression data

GrapHpad Prism5 software was used for statistical analysis of data. In this software, the expression of these genes in the treated strains was compared with the control strains based on 2-ΔCt treated with the control. Significance of data was assessed based on T-test (P≤0.05)

Statistical analysis of data:

Target gene) Ct- (Ct (16srRNA =) untreated group) ΔCt
Target gene) Ct- (Ct (16srRNA =) treated group) ΔCt
(Treated group) ΔCt- (untreated group) ΔCt = ΔΔCt
Fold change = R = 2-ΔΔCt

Data analysis

This study was an attempt to examine Expression of LasR and LasI genes in querum sensing system under the influence of silver nanoparticles modified with silver surface structure in the clinical strain of Pseudomonas aeruginosa. Inthis descriptive study, a comparison of 50 isolates of Pseudomonas aeruginosa strains from different clinical specimens, including blood, urine, wound, sputum secretions, was collected from patients admitted to Milad Hospital.

Results of Pseudomonas aeruginosa biochemical tests

Simon Citrate, Urease, McConkey Agar, TSI, SIM, MR VP tests were used to detect Pseudomonas aeruginosa samples and confirm the authenticity of the isolates. The results were analyzed by the above tests based on (Table 1), so that Pseudomonas aeruginosa samples were identified and available for the next parts of the study.

Table 1: Results of Pseudomonas aeruginosa diagnostic tests

test	
Citrate	(+)Positive
Urea	(+)Positive
SIM	(-)Negative (+)Movement
TSI	K/K
MR	(-)Negative

The Results of Minimum Inhibitory Concentration (MIC) of Silver Oxide Modified Iron Oxide Nanoparticles for Pseudomonas aeruginosa.

According to the experiments, it was observed that the minimum inhibitory concentration of iron oxide nanoparticles modified surface structure with silver in different isolates of Pseudomonas aeruginosa is different. The lowest measured MIC was 0.512 mg / ml in 6 isolates and the highest measured MIC was 80mg / ml in 7 of 50 isolates. 30% of samples were inhibited at a concentration of 20 mg / ml (15 samples).

Table 2: General results of the microbial section

MIC	Number of strains
0/512	6
2	3
4	4
12	10
20	15
60	5
80	7

Results related to the study of 16srRNA gene expression
Standard curve was used to determine the amplification efficiency and PCR efficiency in qR-T PCR technique. To perform this test, the cDNA used was diluted 1 to 5 and the standard curve was drawn according to the intervals of the amplification cycles. The standard curve for the 16SrRNA reference gene shown in the following Figure shows 99% efficiency.
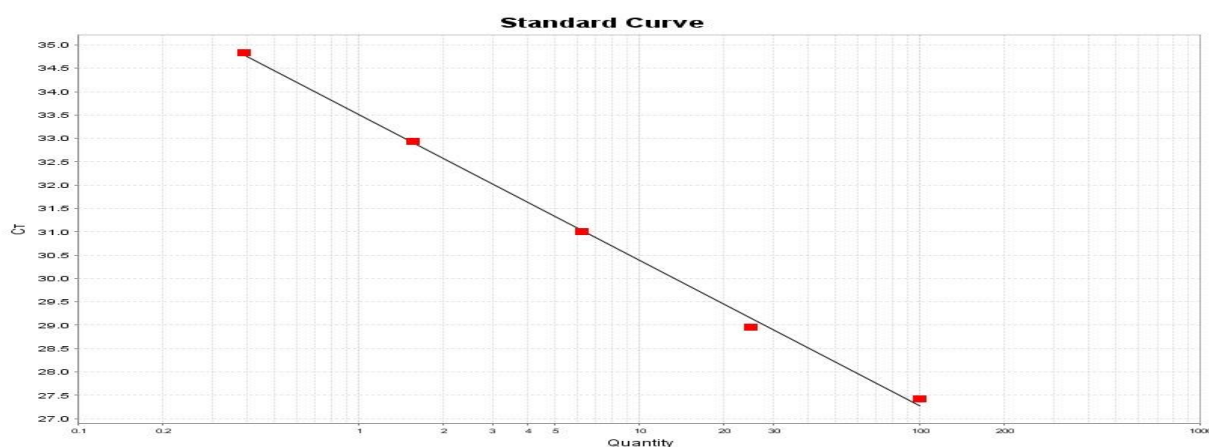

Figure 1: Standard 16s rRNA gene curve

Figure 2: shows the melting curve of the 16srRNA gene. The peak of the curve indicates the specificity of PCR products
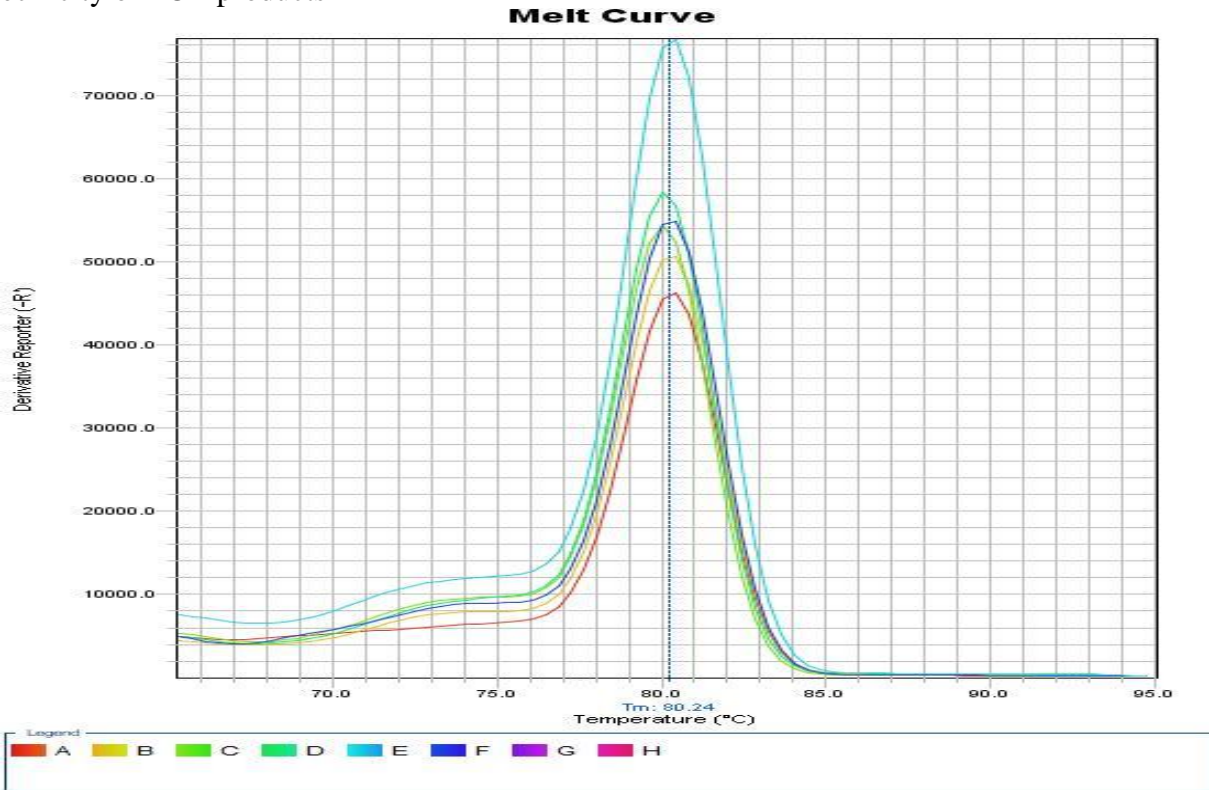

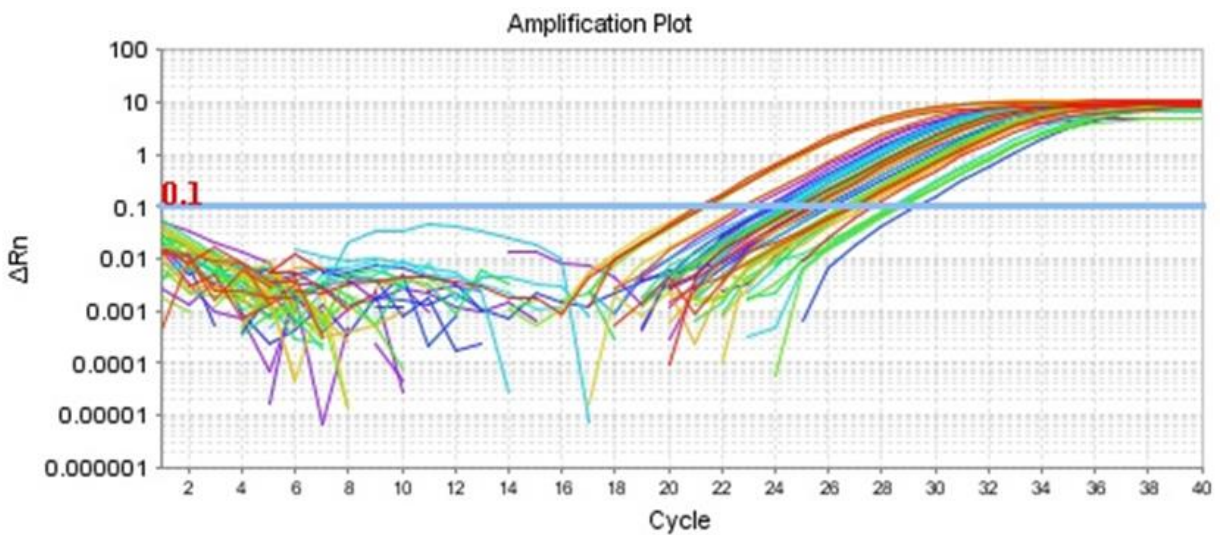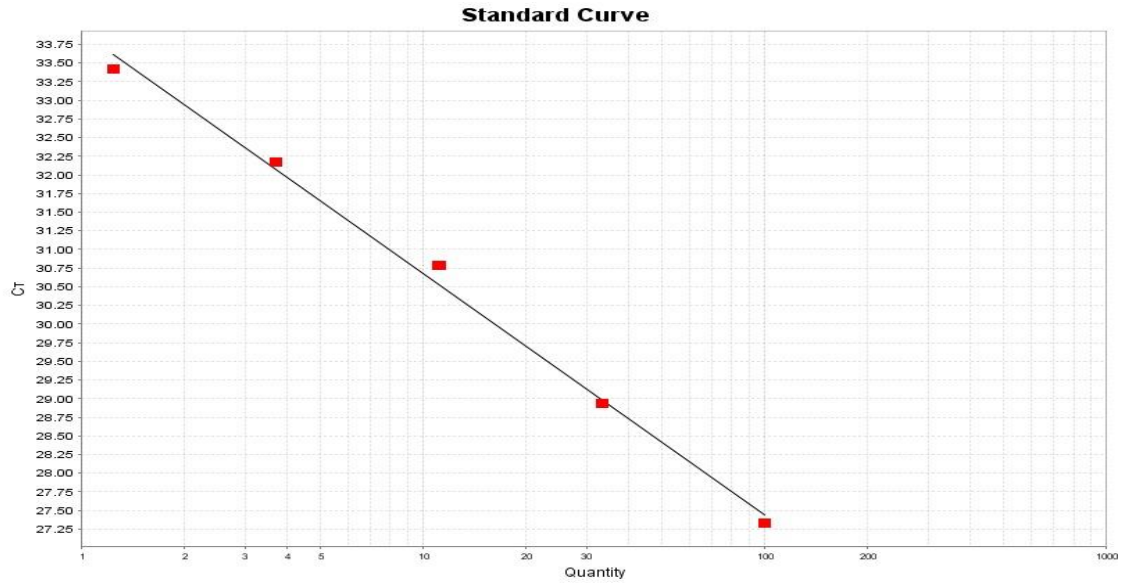Figure 2: Melting curve for the product of 16sRN gene amplification

Figure 3: Logarithmic phase of 16s rRNA amplification

52

Standard LasI gene curve Figure (4). It is acceptable if it is between 90 and 110%

The melting curve of LasI gene is shown in silver .Figure 4: Standard curve of the LasI gen*e* nanoparticle-treated samples of surface-modified silver structure in the following figures (5).
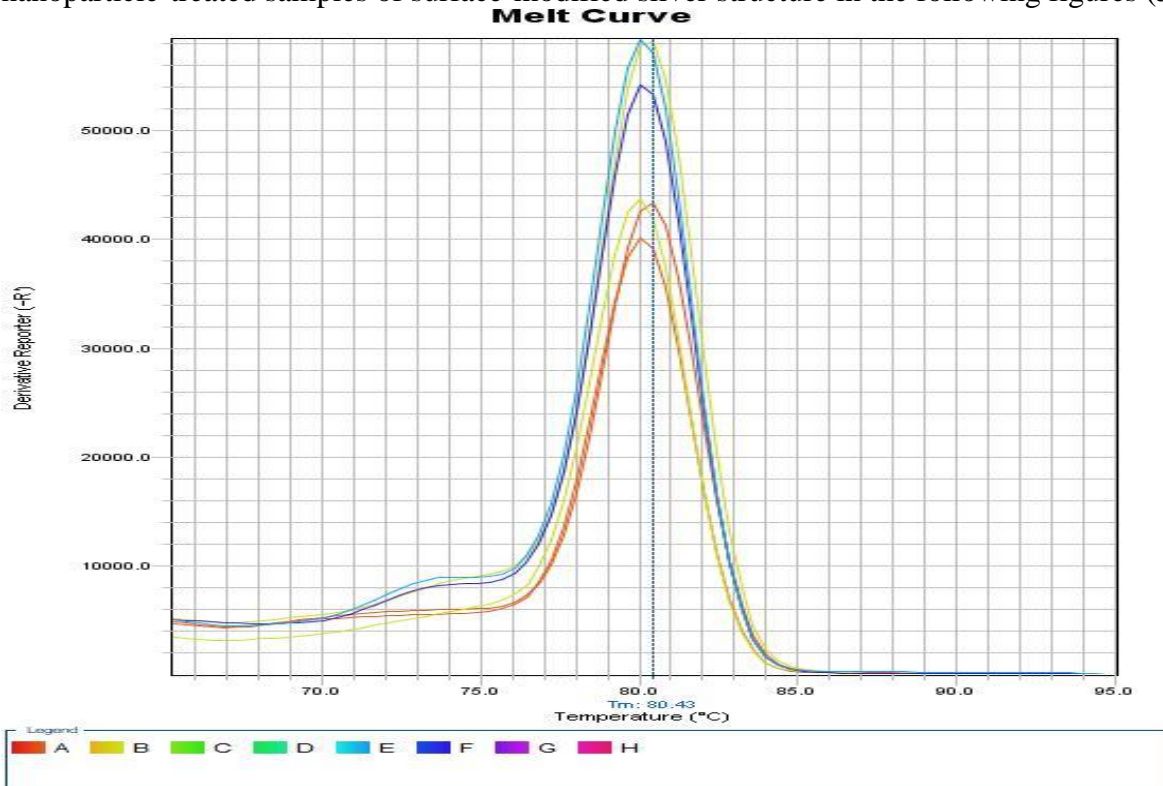

Figure 5: Melting curve for the product of LasI gene amplification

53

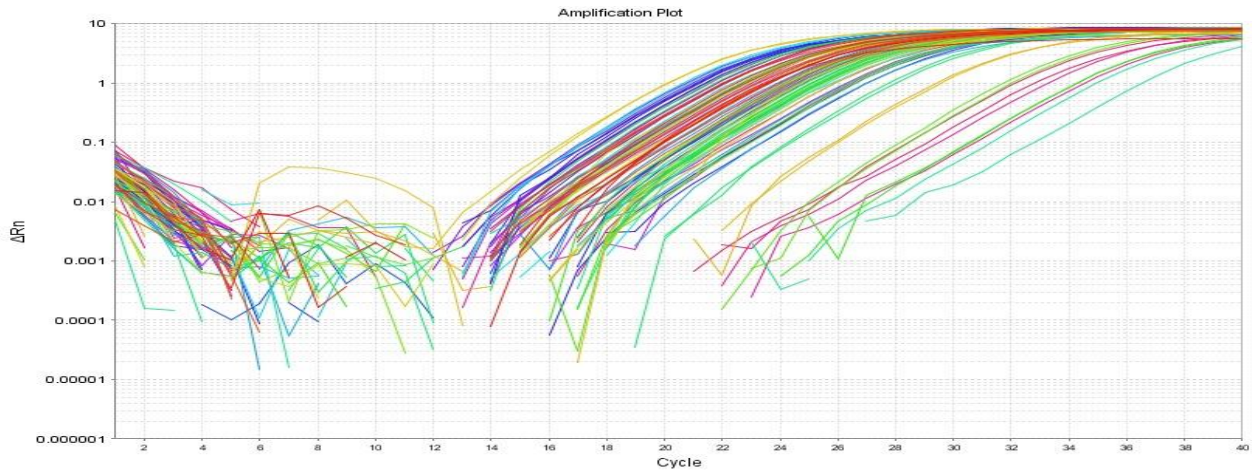The logarithmic phase of LasI gene amplification is shown in Figure

Figure 6: Logarithmic phase of LasI gene amplification

The extent of changes in LasI gene expression in strains treated with silver nanoparticles modified surface structure with silver is examined in Figure7
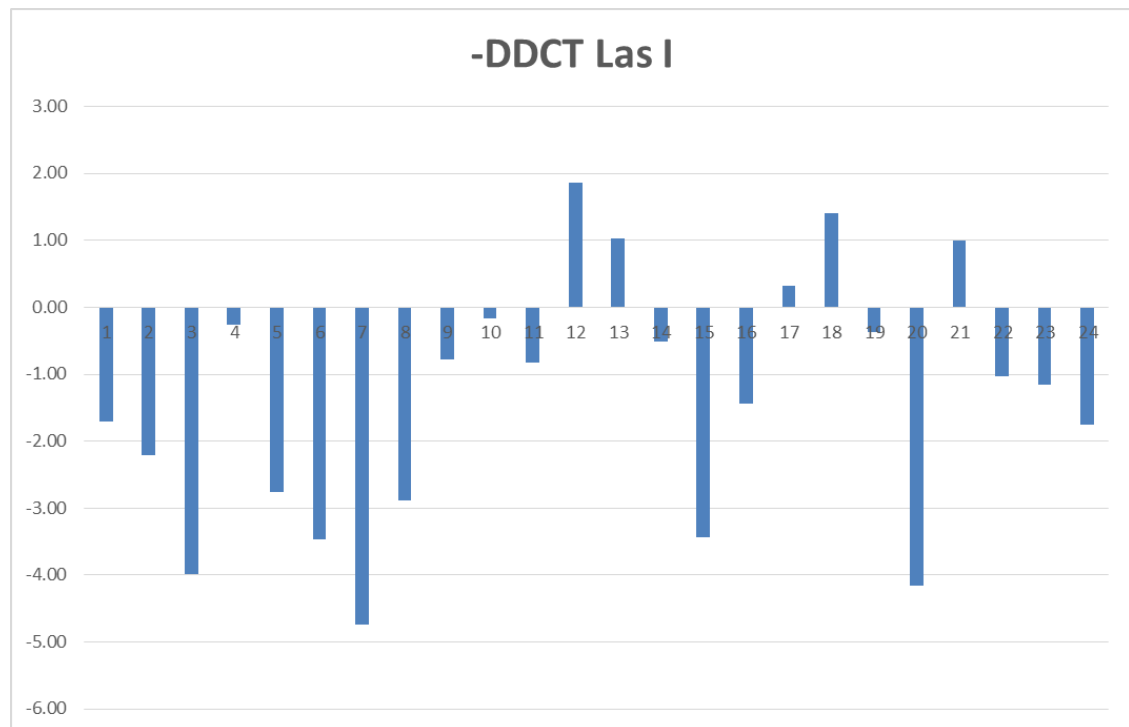

Figure 7: The extent of changes in LasI gene expression in samples treated with iron nanoparticles modified surface structure with silver

54

The mean expression change of LasI gene in treated samples compared to control samples is shown in Figure 8.
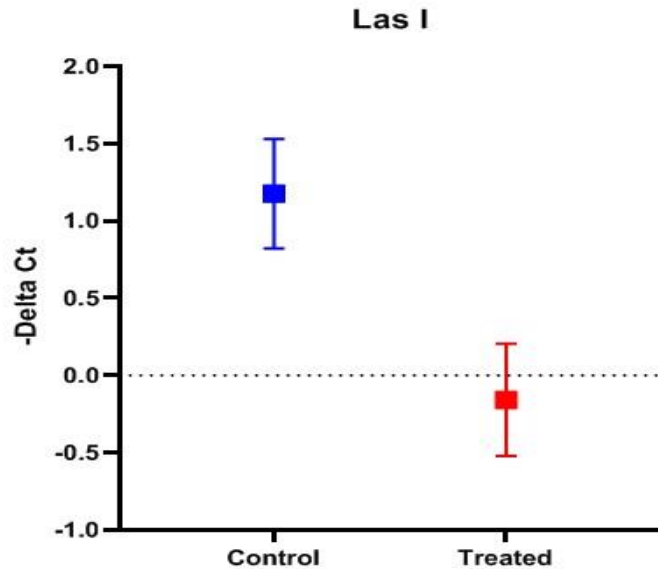

Figure 8: Mean mean change of LasI gene expression in treated samples compared to control As can be seen in Figure 4-8, the expression of LasI gene after treatment with silver .samples nanoparticles modified surface structure with silver was calculated, which is a significant reduction in gene expression (Pvalue = 0.013). The efficiency of PCR, LasR gene, was calculated using serial dilutions of cDNA as follows in Figure 9
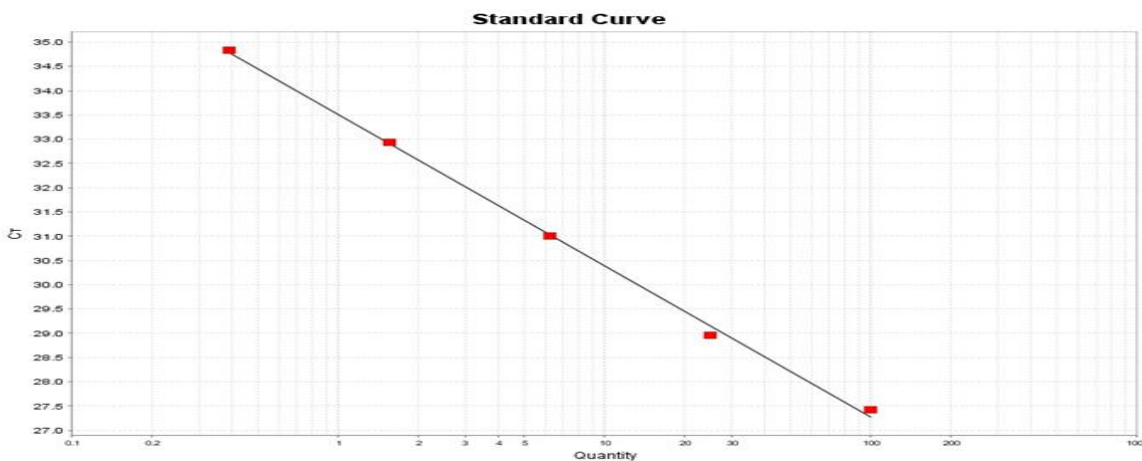

Figure: 9 Standard LasR gene curve

The melting curve of LasR gene in samples treated with silver nanoparticles modified surface structure with silver is shown in Figure 10.
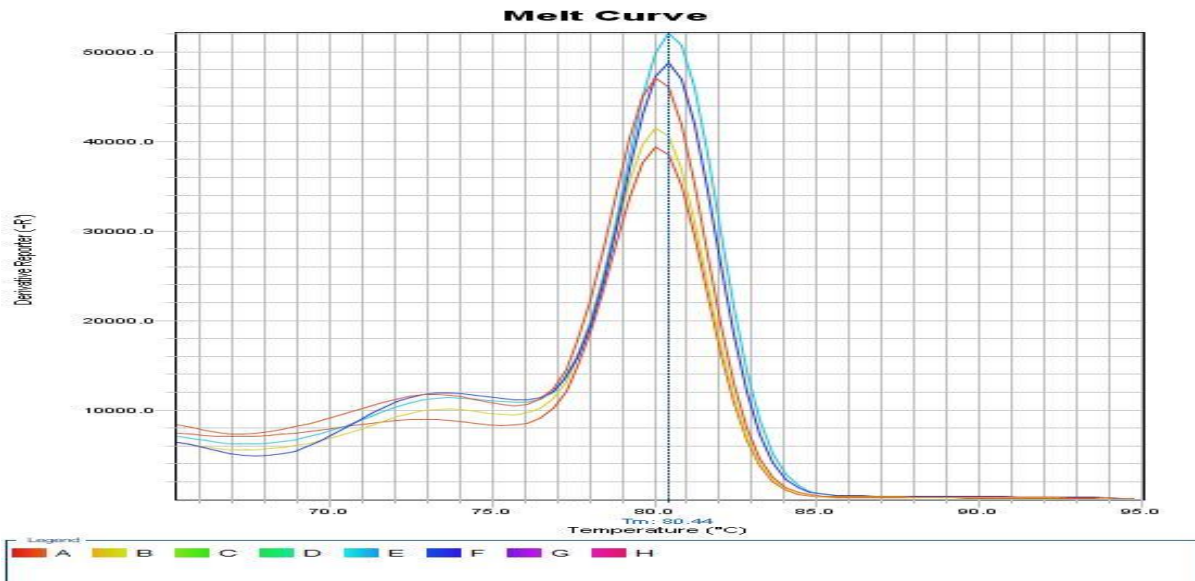

Figure: 10 LasR genes melting curve

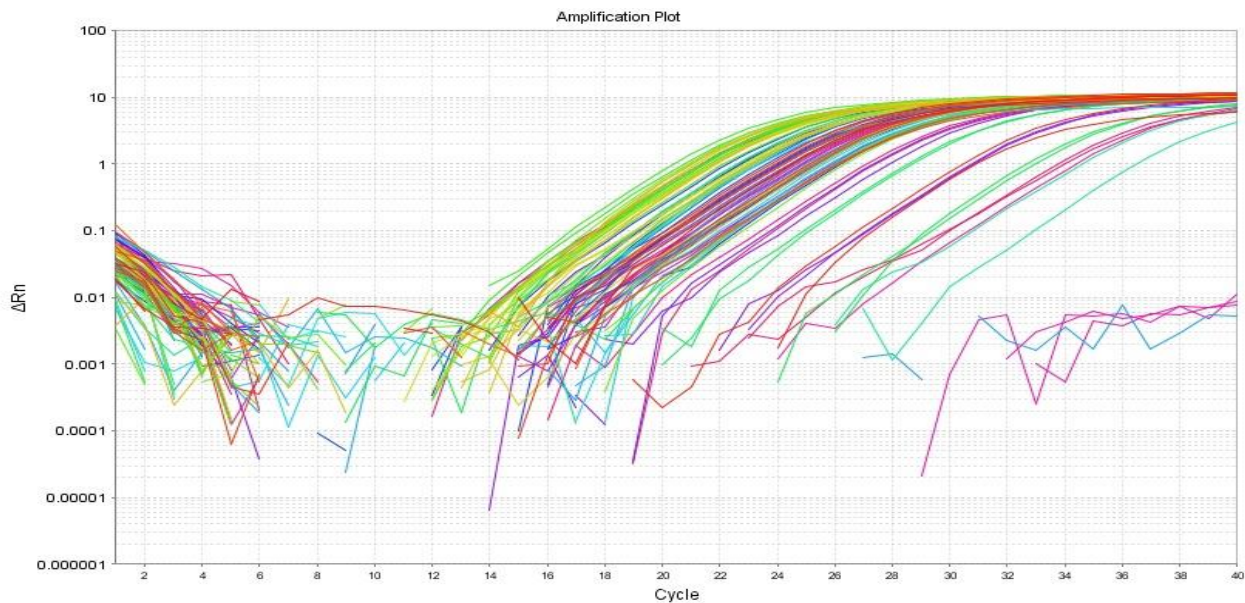The logarithmic phase of LasR gene amplification is shown in Figure 11

Figure: 11 Logarithmic phase of LasR gene amplification

The extent of LasR gene expression changes in strains treated with silver nanoparticles modified surface structure with silver is examined in Figure 12
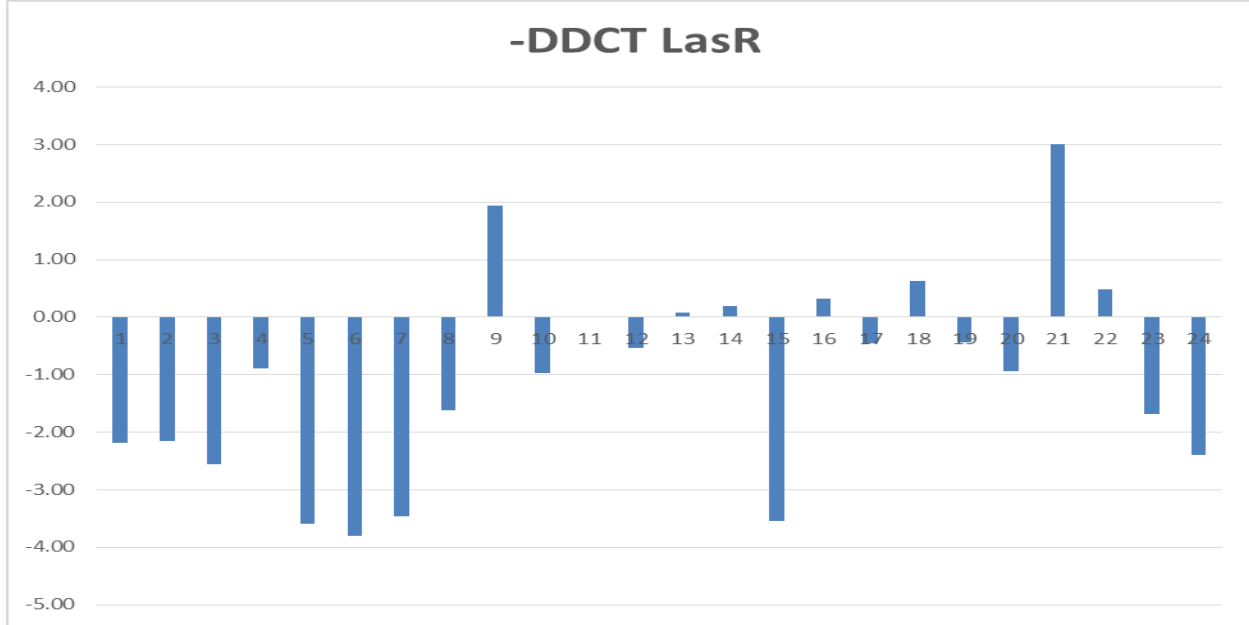

Figure 12: Results of the study of changes in the expression of LasR gene treated with silver nanoparticles modified surface structure with silver

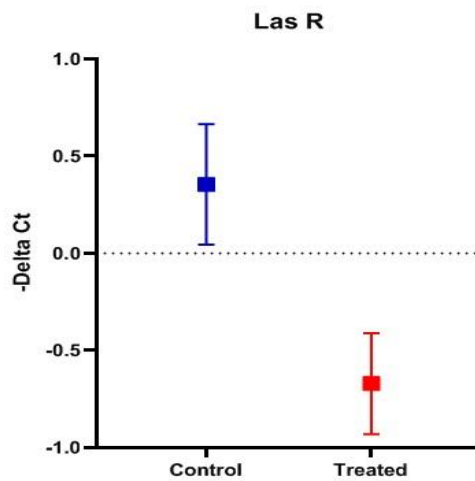The mean change of LasR gene expression in treated samples compared to control samples is shown in Figure 13

Figure 13: Mean change of LasR gene expression in treated samples compared to control samples
As shown in Figure 13,LasR gene expression changes in samples treated with silver nanoparticles modified surface structure with silver were calculated, which is a significant decrease in gene

expression (Pvalue = 0.015). According to the presented data, it was concluded that iron oxide nanoparticles modified by surface structure with silver have an effective role reducing the expression of both LasI and LasR genes.

CONCLUSION AND DISCUSSION

The present study aims at exploring the effect of silver oxide nanoparticles modifying surface structure with silver on the expression of LasI and LasR genes in the formation of Pseudomonas aeruginosa biofilm. Pseudomonas aeruginosa is an opportunistic and pathogenic bacterium that causes the production of pathogens such as lectin, pyocyanin, elastase, raminolipid and their prevalence in hospitals. Inpatients may become infected through contact with the environment. Various mechanisms are involved in the development of resistance in bacteria against antimicrobial compounds and the immune system. Bacterial biofilm is a complex, defensive, rigid structure of bacteria together to form a natural defense barrier, which greatly facilitates the ability to cause infection in a living host. The structural properties of biofilm-forming bacteria are due to the extracellular polymeric material (EPS) that forms the protective shield for intra-biofilm bacteria. Although the physical and chemical composition of EPS varies between species, it contains 50-90% of the total biofilm organic matter and contains exopolysaccharides, extracellular DNA (eDNA), proteins, lipids, and humic substances. The bacteria that make up the biofilm release or suspend their metabolism and laminate well into the polysaccharide and protein matrices. This matrix inhibits the bacteriostatic or bacteriocidal activity of antibiotics. Antibiotics only target floating planktonic cells but are not able to penetrate the biofilm matrix. Published evidence suggests that biofilm formation may also be the result of several other mechanisms, such as cellular signaling or quorum sensing. Quorum sensing is a cellular communication mechanism involving self-inducing producers and regulators. In Pseudomonas aeruginosa, acyl homocerin lactone is the major signaling molecule that controls approximately 300 genes responsible for various cellular functions, including their pathogenesis.

Due to the important role of quorum sensing in the communication between microorganisms and pathogenicity, agents with anti-quorum sensing activity known as quorum quenching (QQ) have promising potential as antimicrobial agents. The antimicrobial capacity of QQ depends more on reducing the pathogenicity than killing the target bacteria. It is believed that this method not only reduces antibiotic resistance, but also improves the treatment of uncontrollable MDR infections. Although collecting information on QQ factors is challenging, due to the dispersed volume of scientific literature, the development of a computational workflow to retrieve and integrate such information has the potential to uncover interesting links and could lead to new insights into Quorum's core therapies.

The antibacterial effect of nanoparticles, including iron oxide nanoparticles, against drug-resistant bacteria and biofilms depends on various factors such as the size of the contact surface of microorganisms and nanoparticles, the type of surface charge of nanoparticles, nanoparticle concentration, nanoparticle size, shape, etc. Positively charged iron oxide nanoparticles showed more antibacterial activity than negatively charged iron oxide nanoparticles. It seems that the reaction between the negative charge of iron oxide nanoparticles and bacteria is weak due to the

high repulsive force between the nanoparticle and the bacterial surface, and this causes the nanoparticles not to bind to the bacterial surface. On the other hand, the use of high concentrations of iron oxide nanoparticles has shown antibacterial activity.The reaction between nanoparticles and bacteria triggers oxidative stress reactions, inhibition of enzymes, inactivation of proteins and changes in gene expression. The most common antibacterial mechanisms of nanoparticles are related to oxidative stress reactions, metal ion release and non-oxidative mechanisms. The main mechanism proposed to explain the antibacterial activity of iron oxide nanoparticles is the oxidative stress caused by reactive oxygen species (ROS). ROS, including superoxide, hydroxyl, and hydrogen peroxide, and single oxygen radicals, can lead to chemical damage to bacterial proteins and DNA. These elements are natural byproducts of oxidative metabolism in cells and play an important role in regulating cell survival and death, cell differentiation and signaling. In bacteria, reactive oxygen species are produced by their cellular respiration process and their production is balanced by intracellular antioxidants. Increasing the amount of these compounds causes oxidation of biomolecules and cell components and ultimately leads to severe cell damage.

The use of nanoparticle technology to control pathogens is preferred over other methods due to its high efficiency, practicality, increased capacity, cost-effectiveness, environmental friendliness an In the present study, iron oxide surface nanoparticles with silver structure were used to increase the permeability and antibacterial properties of the nanoparticles. The advantage of the results of this study is that the inhibitory concentrations are very low, so that the highest inhibitory concentration is related to the concentration of 20 mg / ml of nanoparticles, which can be due in this study; real-time pcr method was used to study the expression of LasI and LasR genes. In order to study the expression, the expression of these genes was compared between samples treated with iron oxide nanoparticles of silver-coated surface with control samples. The reason for choosing LasI and LasR genes was that the expression of these two genes on the production of disease factors affects the generator.

In this study, real-time pcr method was used to study the expression of LasI and LasR genes. In order to study the expression, the expression of these genes was compared between samples treated with iron oxide nanoparticles of silver-coated surface with control samples. The reason for choosing LasI and LasR genes was that the expression of these two genes on the production of disease factors affects the generator. The results showed that after treatment with iron nanoparticles, the surface structure modified with silver had a significant decrease in the expression of both genes.

The findings of the study revealed that iron oxide nanoparticles modified by surface modification with silver with an inhibitory effect on the Las system drastically reduce the expression of both its major genes, LasI and LasR. Due to the control effect of this system, the expression of Rhl genes, ie RhlI and RhlR, is reduced. Therefore, this nanoparticle can be used for medical applications in industry as well.

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