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The Effect of Silver Nanoparticles Manufacturd by Lactobacillus Bacteria on Some Bacterial Isolates Causing Gingivitis

Huda Natiq Faris[1], Bushra Ali Ghyadh[2], Rana Talib AL-Muswie[3]
[1,2] College of Science, University of Thi Qar, Iraq
[3] College of Dentistry, University of Thi Qar, Iraq

Email: hudana-bio@sci.utq.edu.iq[1], bus.al.p@utq.edu.iq[2] , rana-almusawie@utq.edu.iq[3]

Abstract. A Silver nanoparticles is considered to be able to kill multi resistant isolates due to its little dynamic effect on Microorganisms. The Gram-positive bacterium Lactobacillus bulgaricus was used in this investigation to manufacture NPs and evaluate their efficacy against certain pathogenic strains (Staphylococcus aureus, Staphylococcus mutans). For the production of nanoparticles, pure cultures of Lactobacillus bulgaricus were obtained from patients with gingivitis and dental caries and cultivated in "nuterient agar" broth. After centrifuging the Lactobacillus bulgaricus culture, cell-free supernatant containing silver ions was used. aqueous samples, and use a well-agar spread test to assess their antibacterial properties against bacterial strains obtained from the mouths of patients with dental caries and periodontitis. The disc diffusion method is used to create an antibiotic profile that targets certain harmful microorganisms. The main signal of the synthesis and characterization of the silver nanoparticles was a change in hue to a light yellow.The combination is dark brown in hue, as determined by visual and ultraviolet absorption spectroscopy. The results showed that AgNPs generated by Lactobacillus bulgaricus have antibacterial efficacy against certain types of bacteria. Compared to the studied antibiotics, our results indicate that Lactobacillus bulgaricus has a tremendous potential for creating AgNPs that are very powerful against bacteria. Red blood cells are not cytotoxically affected by silver nanoparticles at concentrations of 1.5 mg/ml

Highlights:

1. Silver nanoparticles show antimicrobial potential against resistant bacteria.
2. Lactobacillus bulgaricus synthesized AgNPs tested via disc diffusion method.
3. AgNPs demonstrated strong antibacterial effects without cytotoxicity at 1.5 mg/ml.

Keywords: A Silver nanoparticles; Lactobacillus bulgaricus, pathogenic strains

Introduction

The technological know-how of manipulating and growing nanoparticles is known as nanomedicine [28]. These particles, which range in size from 1 to a 100 nm, showcase specific organic properties that distinguish them from the majority elemental shape [1]. Because of their tiny length, NPs have a larger surface-to-extent ratio than other particles

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of the identical composition, which will increase their catalytic and biological activity. [13].

Recent years have visible a discernible upward push in the use of nanotechnology in a lot of fields, inclusive of biotechnology, agri-meals, engineering, cosmetics, and textiles. It is probably known as a technological revolution. (2013) Perez-Esteve et al. With a focus on meals and fitness, nanotechnology is utilized in medication transport and nutrition release systems (nanoencapsulation), which accelerate the identity of sickness symptoms and provide well timed remedy. It will also be used to use fertilizers and nanoscale additives, or to create nanoscale sensors to stumble on chemical, bacterial, or viral contamination in vegetation. In the food processing region, it's far a unique but promising technique. Moreno-Arribas and Montage (2016).

Because nanoparticles (NPs) have one-of-a-kind physio-chemical characteristics from regular materials, their production has been investigated for a variety of biological programs in latest a long time [8]. Generally talking, steel nanoparticles have many blessings, along with reduced warmness loss because of doping, resistance to corrosion, recyclability, quick thermal responsiveness, and adjustable optical homes [35]. Because of its inherent antibacterial and wound-healing qualities, nano-silver exhibits exceptional promise in therapeutic applications among the many metallic nanoparticles (NPs)—gold, copper, iron, titanium, and zinc [2].

One of the most critical steel nanoscale substances, silver nanoparticles (AgNPs) have sturdy antibacterial features against a number of pathogens, such as oral micro organism [18]. AgNPs have been shown to fight dental biofilm and decrease the prevalence of periodontal disease [26], dental caries [22], and other oral bacterial problems [14] in organic domains which include dentistry. However, there's little evidence to help the antibacterial homes of AgNPs towards medical dental biofilms associated with teeth caries and periodontal sickness. According to Porenczukl et al. (2019), the primary element affecting the activity of silver nanoparticles is their nanoscale, which alters the quantity of silver ion release and disturbs the surface energy. Because of their big floor place, which allows tremendous contact with microorganisms, nanoparticles have strong antibacterial effects in comparison to different antimicrobial therapies. [29].

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The aim of this study was to synthesize and characterize AgNPs, to determine their ability to inhibit growth of bacteria isolated clinically from young and young patients with active dental caries and periodontal disease. The antibacterial and antioxidant residences of silver nanoparticles are frequently connected to them [9].

Methods

Synthesis of Silver Nanoparticles AgNPs

A loop entire natural culture of Lactobacillus bulgaricus become produced, inoculated in MRS broth, and incubated at 37 °C for the complete night time. Following incubation, the biomass become centrifuged for 10 mins at 5000 rpm, transferred to a sterile box, and saved at 4 °C for later use inside the production of silver nanoparticles. The manufacturing of AgNPs used silver nitrate (AgNO3) as a precursor. Ten milliliters of 1 mMAgNO3 solution have been combined with one milliliter of cell-loose supernatant. To generate the reaction, the fluid turned into incubated for the whole night time at room temperature. Ten milliliters of AgNO3 and ten milliliters of distal water have been additionally prepared as manipulate solutions for comparison. The synthesis of AgNPs changed into diagnosed by means of changes in the solution's shade.

AgNPs characterization and formation determination

Silver nanoparticles have been produced and characterised the use of analytical strategies. Visible spectrophotometry, on occasion called spectrophotometer, is a broadly used technique for identifying and detecting silver nanoparticles. A spectrophotometer became utilized to evaluate the solution for a discount research after colour changes within the answer were apparently seen. After the incubation duration, 2 mL of the response combination become measured using a UV-1600 UV Spectrophotometer made by using Shimadzu Corporation in Japan to decide the absorbance spectra (190-650 nm). Using a scanning electron microscope and traditional process, the size and morphological characteristics of AgNPs had been ascertained. Using an EDS (Braker, Netherlands) coupled to a scanning electron microscope with a voltage of 10 kV and a gap length of 5.2.4, elemental analysis for the presence of AgNPs changed into performed in accordance with trendy method.

Silver nanoparticles antibacterial characteristics Cell-unfastened supernatant containing AgNPs changed into evaluated for antibacterial effectiveness in opposition to

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numerous pathogenic lines on Mueller-Hinton (MH) agar plates using the agar nicely diffusion assay. The selected pathogenic bacterial traces, Staphylococcus aureus and Staphylococcus mutans, were acquired from individuals with gingivitis and dental caries. After these strains had been revived from upkeep vials onto MH agar plates and incubated for a complete night time, MH broth changed into prepared in three separate check tubes, inoculated with a different stress in each take a look at tube, and incubated for a further night. 50 µL of AgNPs (1, and a pair of mM) answer and 50 µL of bacterial cell suspension have been introduced to wells of 6 mm diameter that were bored out of MH agar plates in coaching for the take a look at. The sector of inhibition was then measured after the plates had been incubated for the whole night at 30 °C.

Cytotoxicity assay:

Human red blood cells (RBCs) were assessed the use of a modified version of Xian-guo and Urasella's (1994) method. The procedure started with the creation of the blood solution. Using DMSO as a solvent, 1 milliliter of blood became added to 20 milliliters of sterile phosphate buffer saline PBS solution to create lots of extract concentrations (mg/ml). After adding one hundred µl of each concentration to 2 ml of blood solution, the tubes have been incubated at 37 °C. The solution become monitored after three and twenty-four hours to see if the hydrolysis of crimson blood cells led to a murky blood solution, which might imply cytotoxicity, or whether the solution became clean, which would indicate a successful end. In order to compare the outcomes, controls were applied concurrently: a superb manage (blood solution with water) and a negative manage (blood answer with phosphate buffer).

Result and Discussion

Identification and characterization of Lactobacillus bulgaricus

For the motive of producing AgNPs extracellularly, the bacterial strian Lactobacillus bulgaricus turned into isolated from patients. The colony's morphological capabilities revealed that it became round, creamy grey, and had a excessive elevation and complete area. Biochemical exams discovered that Lactobacillus bulgaricus turned into nice for mannitol fermentation and negative for urease, catalase, citrate, and indole. Synthesis and description of AgNPs. AgNPs were efficaciously created by means of inoculating 1 mL of inoculum in 10 mL of a 1 mM silver nitrate answer (1:10). Over the

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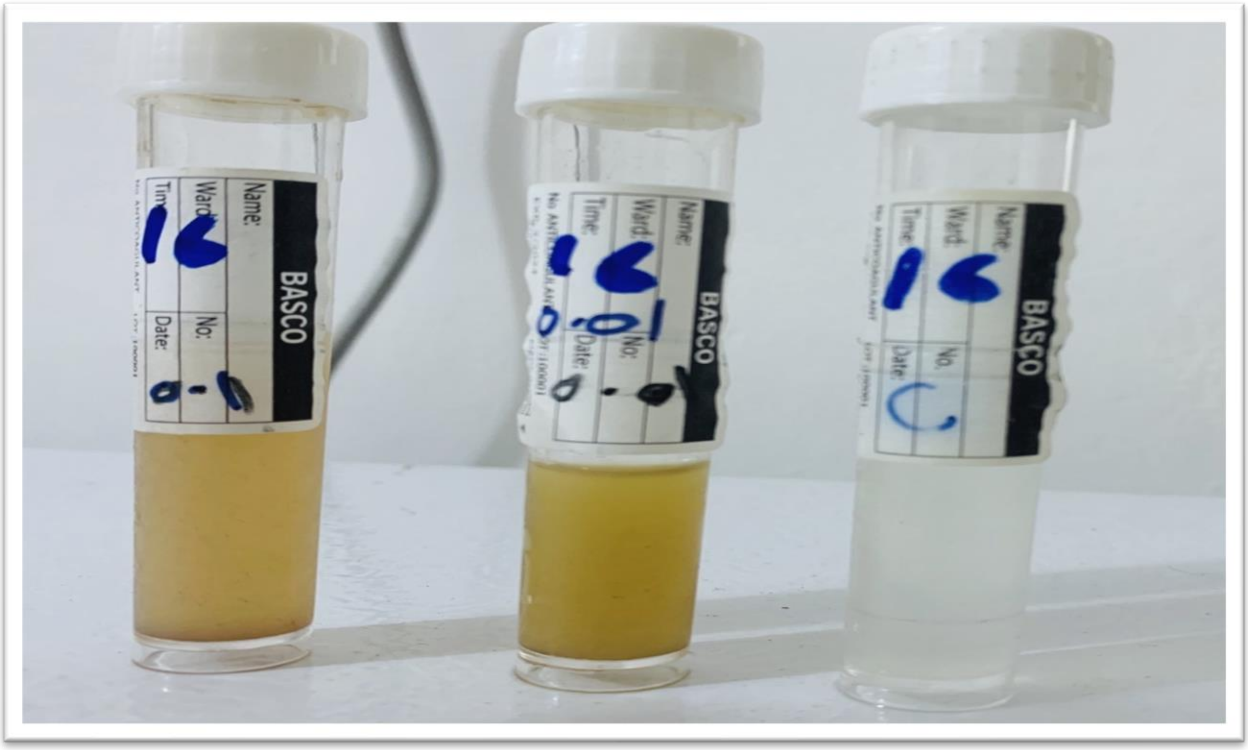
course of the incubation duration, shade adjustments from yellow to darkish brown and an increase in intensity advised that the biosynthesis of AgNPs have been effective Whereas, both controls were observed unchanged. (Figure 1).



2mM

1mM

Control

Figure (1) Biosynthesis of silver nanoparticles at concentrations(2 ,1)Mm and compare with control

Different suspension solutions of AgNO3 with variable concentrations (1-2%) had been introduced to Lactobacillus suspension as a way to ensure accuracy within the effects. Adding varying amounts of AgNO3 to the supernatant has a massive effect at the homes of the produced nanoparticles. At 2 mM AgNO3 and 60º, the maximum amount of nanoparticle production turned into determined (Figure 2).

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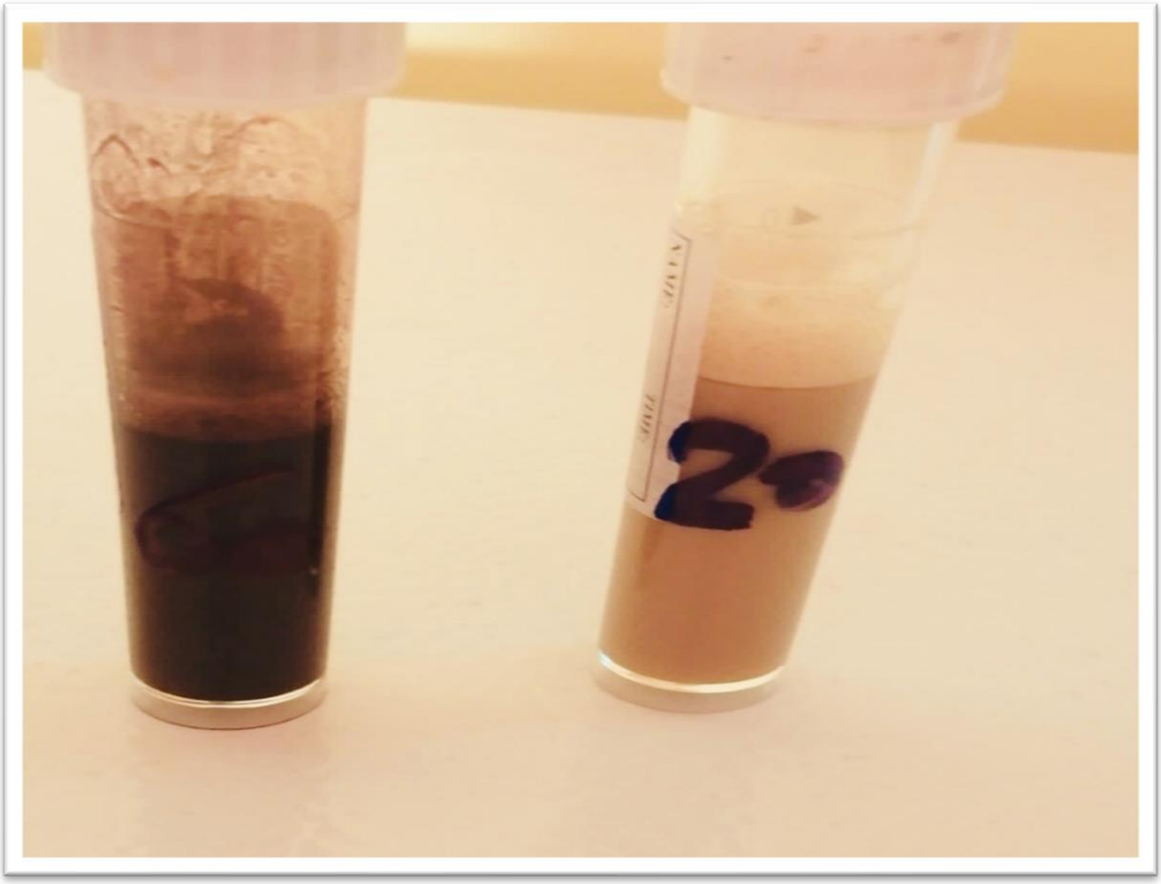

Fig (2) Different temperatures were selected (20 and 60) to produce silver nanoparticles by Lactobacillus in the present study.

Determination of AgNPs

Through UV-VIS absorption, the synthesis of AgNPs become established. Lactobacillus bulgaricus AgNPs had been detected through UV-VIS within the visible range of 190 to 400 nm (Figure 3), which is notion to be the height for AgNPs as previously suggested by using Sikder et al. (2018).

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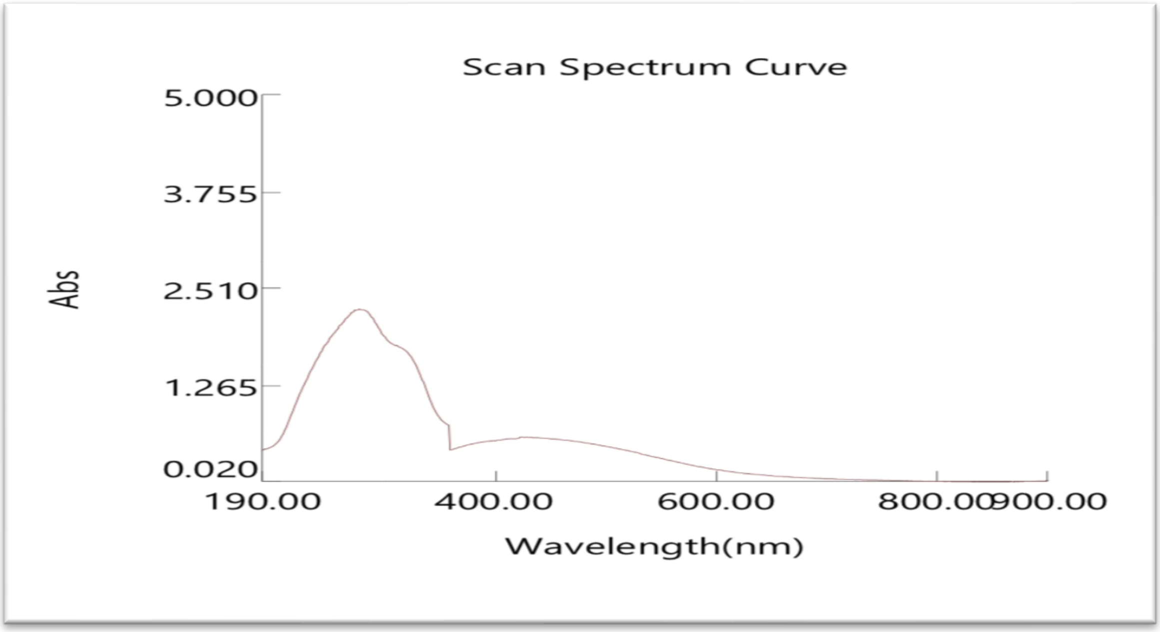

Fig (3) UV-Vis absorption spectrum of Ag NPs

AgNPs sizes and paperwork were ascertained by way of SEM examination, and sphiral-fashioned scattered AgNPs with diameters ranging from 26 to 31 nm have been seen (Figure 4). Adding one-of-a-kind concentrations of $AgNO3$ to the supernatant has a great effect at the homes of the produced nanoparticles. This studies discovered that 2 mM $AgNO3$ changed into the highest quality attention for nanoparticle formation..

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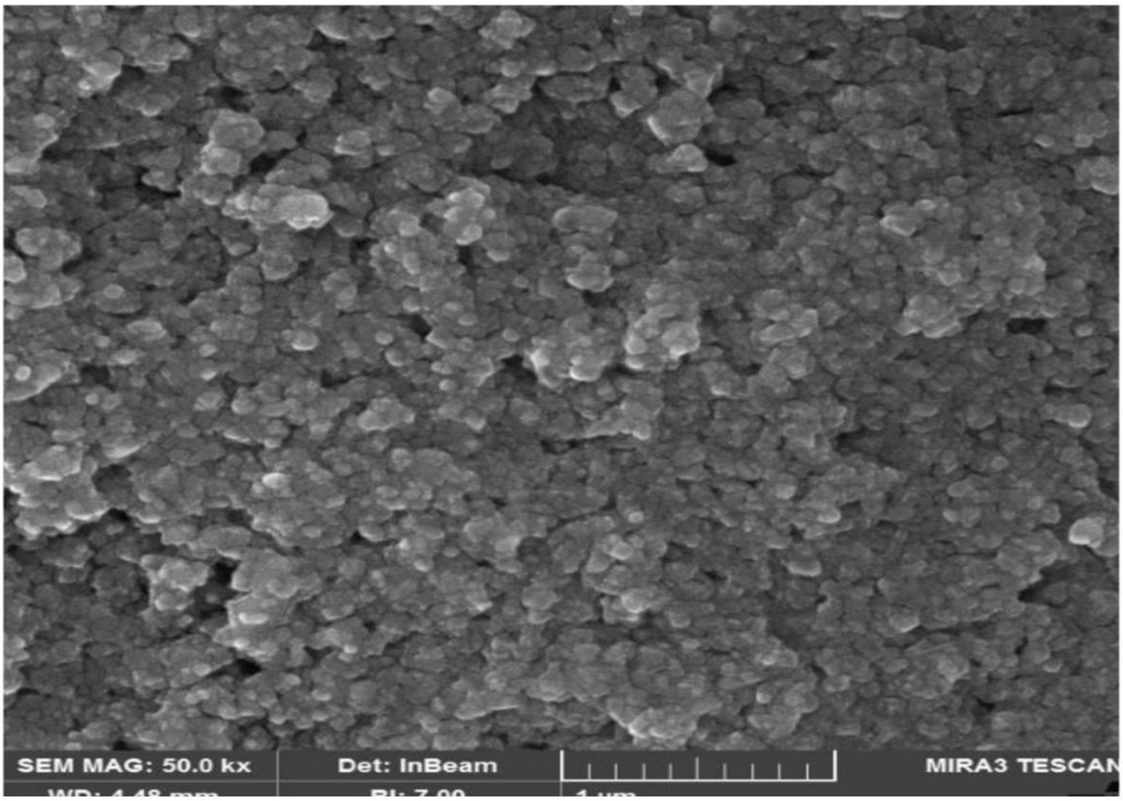

Fig (4) Characterization of the biosynthesized Ag NPs by the analysis of Scanning Electron Microscopy (SEM).

In-vitro Antibacterial Activity of silver nanoparticles (Ag NPs) against Bacterial Isolates

According to the findings, Ag NPs biogenic may stop S. aureus and S. mutans from growing, as shown in fig. (5). The zone of inhibition revealed the lowest inhibition at a concentration of 1 mM, while the highest inhibition was seen at a dosage of 2 mM..

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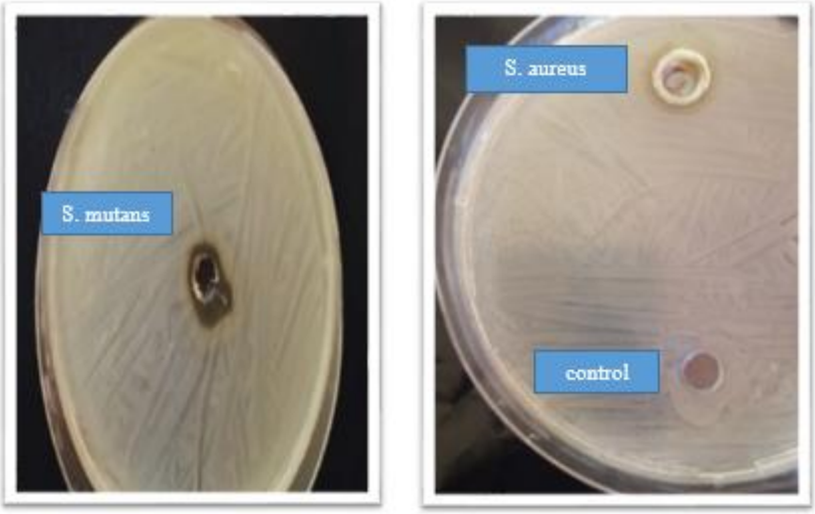

Fig (5) The antibacterial activity of different concentrations of Ag NPs (1.2 mM) against Streptococcus mutans and Staphylococcus aureus was tested.

Cytotoxicity:

Silver nanoparticles did not appear at a concentration of 500 ,100 mg/ml, no cytotoxicity towards red blood cells fig (6) . Non-toxic to cells It is very important, especially the host, when used to prepare or enter sterile or disinfectant materials as one components of chemical drugs (2000, WHO).

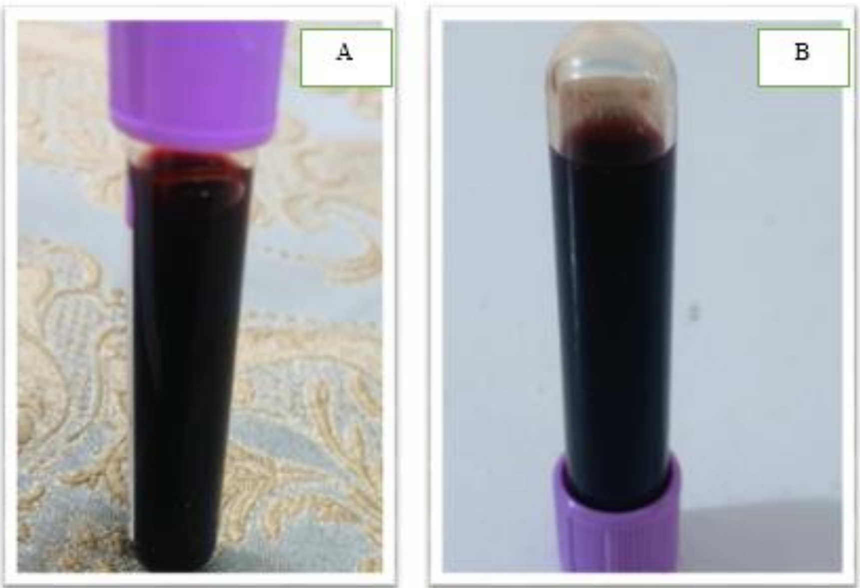

Fig (6): Cytotoxicity towards red blood cells ,control tube (A). after 24 hour with added silver nanoparticles (B)

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Discussion

According to this studies, AgNPs may additionally significantly suppress the bacterial proliferation of microorganisms found in clinically acquired dental plaque biofilms from sufferers. Researchers' interest has these days been directed to the employment and evaluation of microorganisms as prospective assets for nanoparticle synthesis.

Gingivitis is an terrific technique to so as to isolate Lactobacillus. The remoted bacteria within the present test were Gram-tremendous, able to thriving at a broad range of temperatures and metabolizing various carbohydrates. The end result and the functions (Bergie's Handbook of Specific Bacteriology) have been in comparison. The remoted bacteria were determined to belong to the genera Staphylococcus aureus and Staphylococcus mutans. According to analyze, AgNPs coated on human dentin and different metal clinical implants might also effectively prevent biofilms from developing at the dentin's and the implants' surfaces and manipulate bacterial proliferation in their proximity [3], [4]. Lactobacillus species have previously been hired for the production of AgNPs, however about. In the cutting-edge investigation, Lactobacillus bulgaricus stress changed into validated as fine for biosynthesized silver nanoparticles as medium colour adjustments from yellow to brown .

Lactobacillus bulgaricus serves as a lowering agent, lowering silver from the ionic shape Ag^+ to Ag^0. In a separate studies, after incubation Lactobacillus bulgaricus for the whole night, a brown mass collected around the flask's base. Additionally, it has been suggested that an enzyme called nitrate reductase was responsible for lowering silver from an oxidized nation to a highly reduced one. In standard, this enzyme became launched into the answer and converted silver nitrate to silver nanoparticles with the assistance of capping factors (such as protein) [7]. Numerous research have already been finished wherein reducing retailers, consisting of plant extracts and sunlight, are employed to biosynthesize AgNPs. The glycolipid bio-surfactant isolated from Pseudomonas sp. Turned into also hired as decreasing agent [6], [22]

In our studies, The UV-VIS absorption spectra of the reaction aggregate of Lactobacillus bulgaricus proved the synthesis of AgNPs, and the measured peak within the seen area ranged from 190 to four hundred for the AgNPs, which is the anticipated top for AgNPs. The outcomes have been similar to the ones of another take a look at

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that hired UV-visible spectrophotometry to discover AgNPs produced by means of Lactobacillus species with absorbance at 410 nm [31]. The size and floor location of silver nanoparticles enable them to reach and establish contact with the microbial mobile's DNA, which accounts for his or her tremendous efficiency. When silver nanoparticles bind to the cellular membrane's pores, they launch silver ions, which increase the membrane's permeability and in the end cause the cellular to die [33]

Furthermore, the enhanced floor vicinity of these nanoparticles allows for extra contact and interplay with the microbial cell. The launch of silver ions from the silver nanoparticles, which remain in the cellular membrane to regulate its structure and eventually raise its permeability, is without a doubt what produces the fatal impact on microbial cells. As he additionally stated, until the cell dies [34]. Not destructive to cells When used to make or input sterile or disinfecting materials as one of the additives of chemical cures, it is crucial, specially for the host [36]

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