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Research Article

Biological Assessment Using Nanoparticles

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Abstract

Objectives: The increase of foods infected with microbes such as grain and stored seeds of the most important things that pose a global threat and there was an urgent need to use effective and safe antimicrobial and the use of nanoparticles to combat microbial pathogens are of increasing interest. In this study was used Aspergillums Niger to reduce Silver nitrate salt in nanoparticle particles (AgNPs).

Methods: Production conditions were 100 milliliters of silver nitrate at 28°C and pH 5.5.

Result: AgNPs the concentration of adsorption at the plasma surface of the silver particles, which was shown in silver color, changed the color of leachate from red to condensed milk after 24 hours of incubation. The filtration without AgNP was found to have no change in color. AgNP was observed by TEM spherical and smooth with sizes of 20-100 nm. "Infrared spectroscopy" was used, and an RI analysis was accomplished. The result was the release of the proteins in the leachate and dictated by the reduction of the silver ions present in the solution. In SEM imaging, direct effects were found on multicellular cytotoxicity and detecting large amounts of *P. aeruginosa* and *S. aureus* cells interacting with AgNPs unlike nature showed a smooth surface.

Biological activity was recorded against *Aspergillums flavus, Candida albicans, Bacillus cereus, pseudomonas aeruginosa, Escherichia, Escherichia coli, Staphylococcus aureus*.

In optimal conditions with a bacterial test that showed low sensitivity to concentrations (AgPNs) with MICs of 5, 10, 11, 8, 7, 7 and 10 ml, respectively.

Conclusion: Here through the inhibitory sub-concentrations of AgNPs the BIO film was successfully formed by bacterial pathogens that were tested and the effect of cytotoxicity and anti-tumor activity. The result confirmed that the sensibility of the human cancer cell line to AgNPs was higher than the Vero cell line at the same dose level of AgNPs.ace.

Keywords: Fungi; biosynthesis; AgNPs; TEM; HepG-2; MCF-7; Biofilm

Introduction

Nanoparticles are known to have at least one dimension of 100 nm [1].

Their chemical composition contains bio-degradable or toxic agents [2,3].

Therefore, environmentally friendly alternatives have been used to produce chemicals such as manufacturing through biological synthesis, sought. Several studies have reported that microorganisms, plants and fungi can create nanoparticles through biological pathways [4-6]. It has been discovered that pathogenic and non-pathogenic fungi are capable of the bio-composition of nanoparticle particles [7].

Many authors have used a variety fungal isolates to produce silver nanoparticles (AgNPs) from silver nitrate salt, both extracellularly and intracellularly Khan., *et al.* [8] and Zhao., *et al.* [9] including (*Fusarium oxysporum*) [10], (*F. semitectum*) [11] (*Aspergillus fumigates*) [12], (*A. niger*) [13], (*A. clavatus*) [14], (*Penicillium brevicompactum*).

Siddiqi., *et al,* [15] (*Cladosporium cladosporioides*). used fungi as a good model for nanoparticle synthesis and is the best among oth-

er microorganisms. and the disorder in its use is complex in growth essentials, but it is easy to manufacture. For fungi, exogenous secretions contain reductive proteins, so nanoparticles are deposited externally and free of materials Which allows for direct use of various applications. [16]. It has been established that AgNPs possess antimicrobial activity [17]. *In vitro* cytotoxicity analysis techniques decrease the use of these tissues and cells that have been increased [18,19]. At present, the biological evaluation of nanoparticles has been carried out using fungi and has given encouraging results against both bacteria, fungi and cancer cells. Currently, our food is more contaminated than it ever was. Due to the increasing use of pesticides, we may find ourselves facing a dangerous threat against mankind. This threat is called Bacteria.

Materials and Methods

Microbial species

A. niger, A. flauvas, candida albicans and Gram-positive bacteria, *B, cereus, Staphylococcus aureus* and Gram-negative bacteria, *Escherichia coli, Pseudomonas* and *S. typhi.* were all collected from the Department of Microbiology at King Faisal Specialist Hospital, Riyadh City, Saudi Arabia.,. These bacteria were utilized for bioactive study of silver nanoparticles (AgNPs) (from 2016 to 2017).

Biosynthesis of agNPs

A. niger was inoculated in 500 ml Erlenmeyer flasks containing 100 ml of MGYP medium, composed (g/L) of malt extract (3g), glucose (10g), yeast extract (3g), and peptone (5g) and incubated at 25–28°C under shaking conditions (200 rpm) for 4 days. After incubation, mycelia were collected from the culture broth by centrifugation at 5000 rpm for 20 min and washed with sterile distilled water. Ten grams of mycelialmass was re-suspended in 100 ml of AgNO3 solution in 500 ml Erlenmeyer flasks at pH 5.5, and shaken at 28°C (200 rpm) for 72 h. The AgNPs bio-transformation was routinely monitored by measuring the solution's change in color Centrifugation was performed at a comfortable temperature range in doors at 14,000 rpm for 30 minutes, after which the AgNPs solution was resuspended in 1ml sterile water.

UV-visible spectroscopy analysis

Changes in absorption of the filtrate were measured after 24h busing a UV-visible spectrophotometer (Milton-Roy Spintronic 1201). Measurements were also performed after several weeks in to check the stability of synthesized AgNPs.

Transmission electron microscopy (TEM)

For TEM analysis, a drop of cell filtrate was placed on "carbon-coated" copper grids, and then dried by evaporation at room temperature. A GEOL GEM-1010 transmission electron microscope was utilized to acquire electron micrographs at 70 kV at the Regional Centre for Mycology and Biotechnology (RCMB) [20].

X-ray analysis (EDX)

The existence of elemental silver was established through EDX. using an X-ray microanalyzer, (Oxford 6587 INCA) and a JEOL JSM-5500 LV scanning electron microscope at 20 kV. The EDX spectrum was recorded in spot profile mode from one of the most densely populated silver nanoparticles regions on the film exterior. The Nano crystallites were examined by a Quanta 200 FEG [21].

Scanning electron microscope (SEM)

Bacterial cultures were patronized with AgNPs at concentration of 50 ppm, and incubated for 24h at 30°C. The samples were treated with glutheraldhyde (2.5%) and dehydrated by sequent dilution in ethanol using an automatic tissue processor (Leica EM TP) Next, the samples were dried using a CO_2 critical point drier (Tousimis Audosamdri-815). The samples then "gold coated" SPI-model sputter coater. The samples were exanimated by scanning electron microscopy (JEOL-JSM-5500LV).

Bacteriological assessments

AgNPs were assessed for antimicrobial activity against pathogenic microorganisms.

Disc diffusion assay

Wells were created in nutrient agar plates inoculated with bacteria using sterilized cork borer and impregnated with 100 µL/well AgNPs. Sabouraud Dextrose Agar (SDA), was inoculated with tested fungi before the agar solidification. Wells were cut into the plates using A cork borer, and 50 µL of AgNP solution was dispensed in each well. Negative controls were set up u by sterile distilled water according to the prescribed protocol [22]. Then, plates were incubated at 37°C for 24h for bacteria, and 28°C for 4days for fungi. Microbial susceptibility was estimated by measuring the diameters of the zones of inhibition, with values recorded as the average of three replicates (NARMS, 2002). The percentage of microbial growth reduction (GR%) was estimated using the broth micro dilution method, with the control treatment referenced as: GR%=(C-T)/Cx100. Where, C is the cell concentrations under the control treatment and T is replicates the results were recorded as means ± SE of the triplicate experiment [22].

Determination of Minimum Inhibitory Concentration (MIC)

The lowest concentration of AgNPs resulting in inhibition of bacterial or fungal growth was determined by the broth micro dilution method, and recorded as the MIC [22].

Anti-virulence activity tests

Biofilm Formation and Inhibition Assays

Bacterial strains were tested for their capability to form biofilms by means of the tissue culture plate assay, as described by (Christensen., *et al*. 1995).

The AgNPs was tested for their potential to prevent bacterial biofilm formation at sub-MIC concentration against tested bacterial isolates, according to the method of (Lin., *et al*. 2011).

Evaluation of the antitumor activity

Monolayer microtiter plates of HepG2 and normal Vero cells were obtained from The Egyptian Organization for Biological Products and Vaccine Production (VACSERA). The monolayer cells were treated with 40, 50, 60, and 70 ppm AgNPs in fresh conservation medium and incubated at 37°C. The microtiter plates were then stained with crystal violet [23]. The number of the surviving cells was determined using an ELISA reader (Sun Rise, TECAN, Inc, USA) at 490 nm after well blending. The viability percentage was calculated a1-(ODt/ODc) 100%, where ODt is the mean optical density of wells patronized with AgNPs and ODcis the mean optical intensity of untreated cells. The 50% inhibitory concentration (IC50) which is the condensation required to cause toxic effects in 50% of intact cells, was predestined from graphic plots.

Results

Biosynthesis of AgNPs

A. niger succeeded in reducing silver salt into silver Nanoparticles .AgNP formation resulted in the color of the culture filtrate changing from an intense red to brown after 24 hof incubate, while the culture filtrate without silver nitrate salt did not demonstration any color change (Figure 1). Smooth surfaced spherical AgNP with sizes of 20 to 100 nm were observed by TEM (Figure 1). AgNPs were determined to have a poly dispersity index < 0.450 using Zeta–sizer analysis. Zeta ability showed the surface of AgNPs have positive charges around 35.5 mV.

Infrared spectroscopy analysis

IR analysis was performed to characterize the chemical temple of AgNPs. The filtrate of silver nitrate patronize A.niger with the solution showed the surface plasmon absorption at 280 and 420

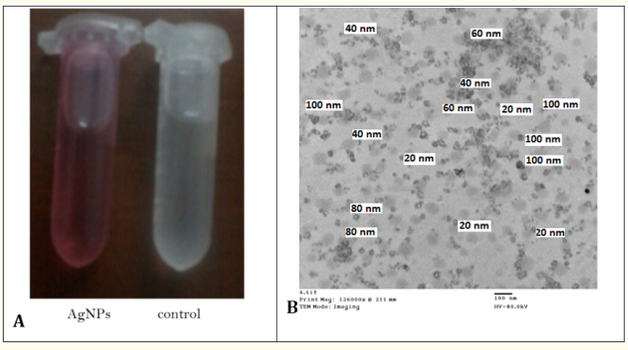

Figure 1: Photo tube gram showing color change observed in fungal extract of A. niger with silver nitrate solution (A). The control without silver nitrate salt did not exhibit any color change after 24 h incubation. Micrograph (B) showing the AgNPs biosynthesized by *A. Niger*. Scale bar = 100 nm using TEM.

SEM imaging

SEM observations confirmed the direct effects of AgNPson cubicle morphology, revelation the effects of considerable amounts of cellular damage (Figure 1) Gram-negative *P. aeruginosa* and Gram-positive *S. aureus* cells treated back AgNPs appeared lean regarding weird stall walls and were surrounded by cellular snippet in comparison normal cells (control) were intact and showed a smooth surface (Figure 2,3).

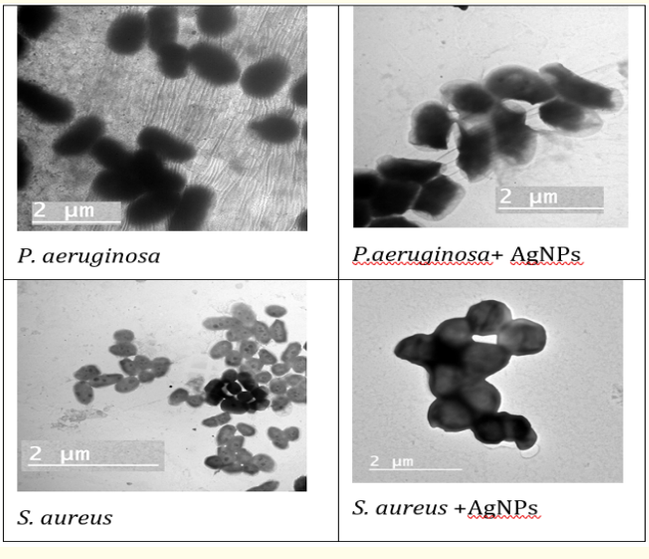

Figure 2: Electro micrograph showing morphological alterations in *P. aeruginosa* and *S. aureus* bacteria exposed to AgNPs. Controls are untreated bacteria. Scale bars are 2 µm.

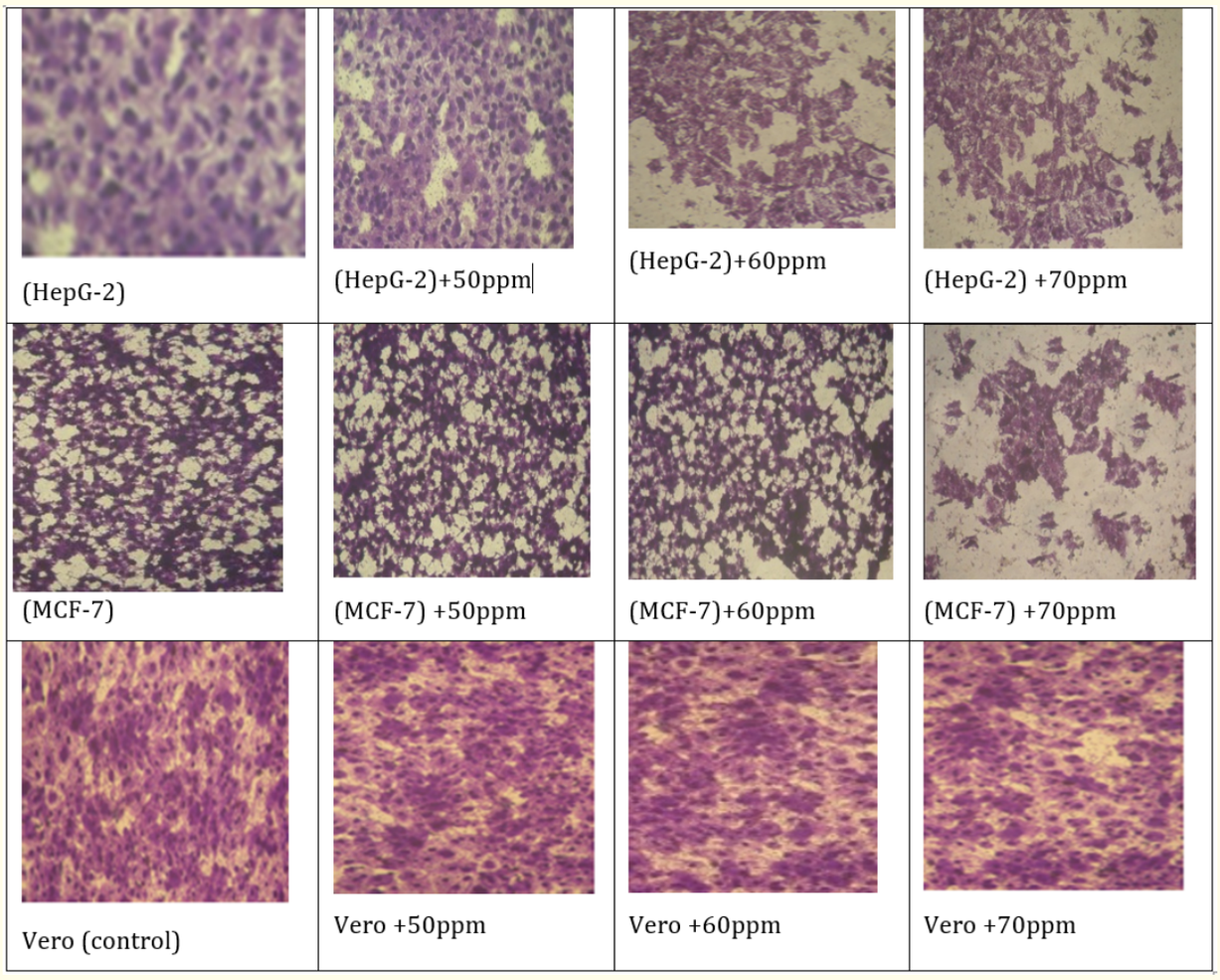

Figure 3: Cell line images, showing effect of AgNPs at different concentrations (C1, C2, and C3) for 24 h on HepG-2, MCF-7 and Vero cell lines comparable to control.

nm was combined with tryptophan and tyrosine residues present in the protein. This result was observed the proteins was released into filtrate and indicated the reduction of silver ions present in the solution.

Antimicrobial activity testing

AgNPs exhibited moderate antimicrobial activity zones of inhibition opposed to *C. albicans* 15.7mm, and Gram-positive *B. cereus* 14.5mm, and S. aureus 13.2 mm, High antimicrobial activity with zones of inhibition was seen opposed to *A. flauvas* 18.5mm, *E. coli* 20.4mm, *P. aeruginosa* 19.2mm, and *S. typhi* 21.2 mm, (Table 1) The median inhabitation was for *A. flauvas* 45.65, and *C. albicans* 74.65, Gram-positive *B. cereus* 75.13 and S. aureus 81.6, Gram-negative E. coli 86.56, P. aeruginosa 92.25, and *S. typhi* 72.15. The MICs of AgNPs 50 ppm opposed to the probed bacteria growth at condensation, *A. flauvas* 5mL-1, C. albicans 11mL-1, Gram-positive *B. cereus* and *S. aureus* 10mL-1, gram negative *E. coli* 8mL-1, *P. aeruginosa* and *S. typhi* 7mL-1.

Amid seven tested microbial isolates, the rugged biofilm producing by isolates *B. cereus* 0.74., *P. aeruginosa* 0.56, *S. aureus* 0.64, and *S. typhi* 0.55, with optical densities. In contrast, moderate biofilmwas featured by *C. albicans, E. coli, P. aeruginosa,* and *S. aureus,* apropos ODs of 0.45 and 0.35, and weak biofilm producing by A. flavus (0.25 O.D.). Biofilm deactivation was investigated for ability to prevent biofilm figuration by powerful biofilm producing bacterial isolates (*P. aeruginosa* and *S. aureus*) grown with far different sub (MICs) concentrations. The results showed that deactivetion of biofilm formation by AgNPs of *A. flauvas* 75.45, *C. albicans* 70.25, gram positive *B. cereus* 64.72, and *S. aureus* 87.23, gram negative *E. coli*

Microbial isolates							
	fungi		Gram positive		Gram negative		
Assessments	*A. flavus*	*C. albicans*	*S. aureus*	*B. cereus*	*E. coli*	*Ps. aeruginosa*	*S. Typhi*
GI (mm)	18.5	15.7	13.2	14.5	20.4	19.2	21.2
MGI	45.65	74.65	81.6	75.13	86.56	92.25	72.15
MICs (mL/L)	5	11	10	10	8	7	7
Biofilm formation	0.25	0.45	0.64	0.74	0.32	0.56	0.55
Biofilm reduction (%)	75.45	70.25	87.23	64.72	67.85	78.44	75.50
Chi-Square			0.18				
DF			2				
P-Value			0.9146				

Table 1: The diameter of zones of inhibition, mean growth inhibition percentages, MgI and Minimum Inhibitory Concentrations of AgNPs MICs against pathogenic bacteria and fungi. AgNPs were applied at 50 ppm (2 mL/L). Each value is the mean of 3 replicates. by ELISA plate reader.

67.85, *P. aeruginosa* 78.44, and *S. typhi* 75.50% at the condensation tested (Table 1). Biological activity counted by diameter zones of suppression was registered against these organisms; *Aspergillus flavus, Escherichia, Candida albicans, Bacillus cereus, pseudomonas aeruginosa, Escherichia coli, Staphylococcus aureus*. Figure 4 and 5, (Table 1) shows the shows the summary of the biological activity, overall gram-positive gram bacteria had the highest reduction percentage. No effect of organisim was recorded ($P < 0.05$).

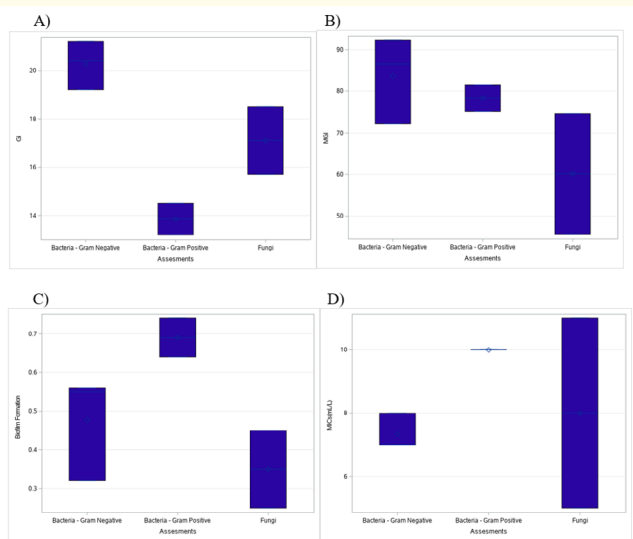

Figure 4: Boxplots of the diameter zones of inhibition by pathogens type for; A) Mean growth inhibition percentages, B) MGI and Minimum Inhibitory Concentrations of AgNPs, C) Biofilm Formation, D) MICs(mL/L).

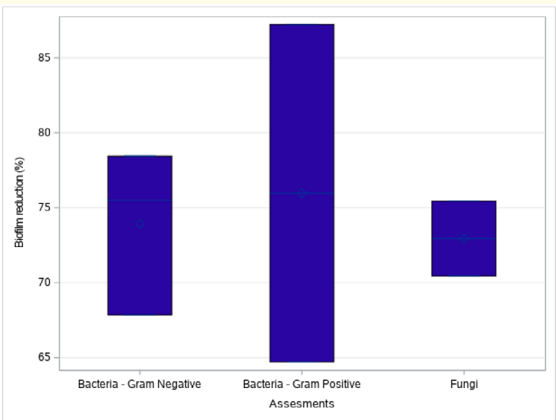

Figure 5: Boxplots for the biofilm reduction percentage by pathogen types. Gram positive bacteria has the highest reduction percentage, followed by gram negative, and lastly fungi.

In optimal conditions with bacterial testing showed weak sensitivity of condensation (AgPNs) with MICs of 5, 10, 11, 8, 7, 7 and 10 ml respectively. The inhibitory sub-concentrations of AgPNs successfully form the BIO film by bacterial pathogens that have been tested. The anti-tumor and cytotoxic effect of the cytotoxicity signal results in the case related to the cell of cancer at the same dose level. Overall the most sensitive cell lines to AgPNs was MCF-7, followed by Hep-G2 and vero cell lines (Figure 6 and Table 2).

AgNPs conc. (ppm)	Viability %		
	(HepG-2)	(MCF-7)	Vero cell
70	40.75	25.85	90.75
60	70.25	65.45	95.25
50	75.65	70.12	100
40	83.36	85.56	100
0	100	100	100
	5 µg/mL	5 µg/mL	-
Cell Lines		Chi-Square	5.12
DF		2	
P value		0.08	
Concentrations (AgPNs)		Chi-Square	7.30
DF		4	
P value		0.12	

Table 2: Antitumor activity of AgNPs on Viability of against (HepG-2), (MCF-7) and Vero cell line.

Human hepatocellular carcinoma cell-line (HepG-2). Breast carcinoma cell-line (MCF-7). African green monkey kidney continuous cell line (Vero clone CCL-81).

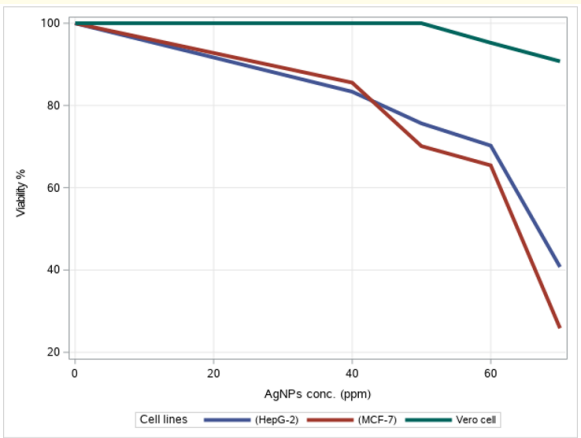

Figure 6: Antitumor activity of AgNPs on Viability of by against (HepG-2), (MCF-7) and Vero cell lines.

Discussion

Currently, our food is more contaminated than it ever was.

Pathogenic bacteria can cause hundreds maybe even thousands of diseases, some of them can't be cured. Furthermore, you can try several ways to get rid of antimicrobial effects of antibiotics used to get rid of them. In this case, we are in a huge need to a new antibiotic which can't be resisted by bacteria, Silver nanoparticles (AgNPs) emerged as replacement to antibiotics because of its distinguished properties.

Silver nanoparticles made by green chemistry offer a potentially new alternative to chemically formed nanoparticles.

In this review, we will talk about the latest developments in the manufacture of nanoparticles, their implementation as antimicrobial agents and the mechanism of antimicrobial action [24].

Generally, the synthesis of nanoparticles has been carried out using three techniques, including physical, chemical, and biological methods. Silver nanoparticles are manufactured using physical methods by condensation and evaporation by an atmospheric tubular heater pressure. The advantages of physical methods are speed, radiation used as reducing agents, and no Evolution in the category of dangerous chemicals, in contrast the negative aspects are: pollution of solvents, high consumption of energy, low yield, and lack of uniform distribution [25].

Chemical methods use water or organic solvents to prepare the silver nanoparticles. This process employs three components, such as metal precursors, reducing agents, stabilizing/capping agents. Physical methods have no major advantage, such as high-yielding chemical methods. Expensive are those methods that I mentioned earlier. Additionally, the materials used for AgNPs synthesis, such as citrate, borohydride, and 2-mercaptoethanol are toxic and hazardous. Ignoring defects, the silver particles manufactured are not the purity required . Moreover, by conducting assembly, excludes many dangerous and toxic by-products out. Recently, Abbasi., *et al*. explained a detailed account of synthesis methods, properties, and bio-application of AgNPs [26].

To overcome the shortcomings of chemical, biological methods have Other solutions have emerged. Recently, biologically-mediated synthesis of nanoparticles has been shown to be simple, cost effective, dependable, approaches and much attention has been given to the high yield production of AgNPs. Bio-sorption of metals by Gram-negative and Gram-positive bacteria provided an indication for the synthesis of nanoparticles before the flourishing of this biological method. Several studies reported the synthesis of AgNPs using green, cost effective, and biocompatible methods without the

use of toxic chemicals in biological methods. In this green chemistry approach, several bacteria, including Pseudomonas stutzeri AG259, Lactobacillus strains, Bacillus licheniformis, Escherichia coli (*E. coli*), Brevibacterium case, fungi including Fusarium oxysporum were utilized. The major advantage of biological methods is the availability of amino acids, proteins, or secondary metabolites present in the synthesis process, the elimination of the extra step required for the prevention of particle aggregation, and the use of biological molecules for the synthesis of AgNPs is eco-friendly and pollution-free.

Maximum nanoparticle synthesis occurred when $AgNO_3$ was supplied at 30.75 mM in the reaction mixture, followed by the addition of 0.5 mM $AgNO_3$. This can be demonstrating on the basis of enzyme-substrate kinetics; i.e. the key biomolecules active site which causes the process of reduction is previously saturated with the silver ions, and there's no available sites for additional ions to become reduced, and so, no further increase in AgNPs synthesis is possible, despite the presence of additional salt substrate [27].

Optimized parameters resulted in an increase in AgNP size and poly dispersity, as it was also found by Mulvaney [28] and Singh., *et al*. [27]**.** In this study, and at optimized conditions, the enhanced rate of production of AgNPs may be the direct result of the silver ions' effect as a substrate, pH, and temperature on a key biomolecule responsible for the process of reduction that occurs in the aqueous filtrate of *A. niger*. (Guzmáz., *et al.* 2019).

In the present study, silver nanoparticles occur a mild antibacterial activity against *C. albicans, A. flauvas*, and *S. aureus* with large areas of suppression, but antimicrobial activity is high against *B. cereus E. coli, P. aeruginosa,* and *S Typhoid*. Areas were suppression of growth 15.7, 14.5, and 13.2 mm respectively High antimicrobial activity was seen against *A. flauvas*, *E. coli*, *P. aeruginosa*, and *S. typhi*, with zones of suppression of 18.5 , 20.4, 19.2 and 21.2 mm, respectively The mean growth in habitation was 45.65, 75.13, 74.65, 86.56, 92,25, 81.6, and 72.15 OD for *A. flauvas, B. cereus, C. albicans, E. coli, P. aeruginosa, S. aureus,* and *S. typhi* enterica, respectively.

A. niger succeeded in reducing silver salt in nanoparticles, niger with the solution showed that surface plasmon uptake at 280 and 420 nm was combined with tryptophan and tyrosine residues present in the protein.

The MICs of AgNPs (50 ppm) against the tested bacteria growth at concentrations, 5, 10, 11, 8, 7, 10, and 7 mL-1respectively. (Guzmán., *et al.* 2019). It is well known that Ag ions and Ag-based compounds have biological activities [29,30].

Owing to their small size, AgNPs impair the sulphur and phosphorus containing essential macromolecules such as proteins and DNA [31]. Thus, action of AgNPs appears to be a consequence of adherence to and penetration inside the cell of the target cells.

Nanoparticles have been developed as image-capture agents, which can be specifically grouped into tumors and avoid tissue damage. Heat can lead to different types of cell death, depending on many factors, which can activate opposition responses. Some of them are desirable, such as induction of a specific immune response that can help eliminate the tumor, but others are best avoided, such as induction of cell growth and inflammation (Mattos., *et al.* 2014).

Silver ions are used in many types of formulations, and it was recently shown that AgNPs hybridized to amphiphilic hyperbranched macromolecules can be used to create effective antimicrobial surface coatings. The most significant application of silver and AgNPs is in production of medical supplies, such as topical ointments to protect from infection in burns and open wounds.

Newly devised AgNP-coated wound coverings have been innovation in the management of wounds or infections [32].

To stop or diminish infections, a novel generation of bandages incorporating antimicrobial agents, such as silver, has been commercialized. wound coverings impregnated with colloidal silver lead to a good reduction of pathogen-specific alterations in epithelium infection (Mordorski., *et al.* 2017).

The application of silver and AgNPs to infected keratinocytes in a moist healing environment is effective, active compared to wound coverings without silver. Similar outcomes with *E. coli* were attained with AgNPs [33,34]. The enhanced cytotoxicity achieved by AgNPs may be attributed to their size, which assists with penetration into tumor cells.

Other researchers have suggested that AgNPs may interfere with active sites of cellular proteins, subsequently cellular chemistry (Behzadi., *et al*. 2017).

Therefore, it is probable that once they penetrate cells, AgNPs may attack functional proteins of cells, leading to partial aggregation and unfolding of proteins, as has been found in the case of bovine hemoglobin.

Toxicity of silver nanoparticles is concentration, size, and shape dependent (Shashi., *et al*. 2017).

In this study, we used different intervals of time to find out whether the antibacterial effect of GC-AgNps was bacteriostatic or bactericidal.

The antibacterial test was measured by the MIC using different concentrations of GC-AgNps. The MIC for *E. coli* as a model of Gram-negative bacteria was 1µg/mL after 120min of exposure with GC-AgNps and enough to inhibit 99.8% of growth. Therefore, ME at 1µg/mL showed < 2.71 in which the bacteria at this level did not present vi- able cells to count. In contrast, the ABE exhibited that concentrations of 0.1 and 0.01 µg/mL were insufficient to inhibit growth [35-61].

Conclusion

In conclusion, it was found that the silver particles have a positive effect against microbes and cancer cells We reached to this result after the silver particles were extracted and conducted many experiments on them On the other hand, silver particles are harmless to the nature as opposed to other materials for that reason silver particles were popular in the medical field.

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