Preparation of Nanosilver particles via sol-gel chemistry and biological activity against *Cryptococus neoformans*

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ABSTRACT

Powder of silver nanoparticles was prepared via Sol - Gel technique successfully using silver nitrate(AgNO3) that gesture is added to Sodium Citrate, (C6H5O7Na3) as a reducing agent and by using Magnetic Stirrer till mix the solutions and heated then via centrifuge to separate the silver Nanoparticles from solution .It is then dry in an oven at a temperature 40 C° for 24 h.. Structure characteristics was studied in previous study , like (XRD) and atomic force microscope (AFM) examined.

Particle size of nanoparticles was calculated for samples prepared for make the biological activity against *Cryptococcus neoformans*,

Cryptococcus neoformans, isolated have been used to determine the cytotoxicity of nanosilver by treatment of cryptococcal meningitis isolated. The entire drug loading Nanoparticles show enhanced ability to inhibit fungal cells proliferation, where, we saw that highest inhibition zone at 25 mg/L concentration and MIC at 5 mg/L concentration.

Keywords: Nanosilver, biological activity, Sol-Gel technique, *Cryptococus neoformans*

INTRODUCTION

Nanotechnology is a new type of science that refers to understand and control the properties of materials at the nano-scale, one nano-meter (one billionth of meter) is the dimension of a small molecule. At this level, matter exhibits diverse and often amazing properties and the borders between established scientific and technical disciplines fade (1). Nanoparticles are a nano structures have a wide class of substances (metals, semiconductors, superconductors, magnetic materials, biomaterials, polymers, water soluble inorganic and organic compounds (2). All these types of nanoparticles (NPs) are a materials with sizes vary between the order of one nanometer to several tens or hundreds of nanometers. Some times more than 100 nano as found in nano cluster(3) with many variable features as size, shape, surface charge and narrow size distribution(4) . One of the important nanoparticle is silver nanoparticles which have widely and significance antibacterial effect. The amazing features of these articles require a large number of pathogenic bacteria, including antibiotic-resistant strains, as well as many viruses and parasites. The nano silver has an antibacterial pesticide longer the privilege of a deadly virus, terrible natural antibiotic and several miracle cures source (5-10). The medical properties of silver have been known for many years. Silver-based compounds are used in many antimicrobial applications. Nanoparticles have been known to be used for many physical, biological, and pharmaceutical applications therefore, many researches have found that using silver nanoparticles as pharmaceutical or using it as base material to prepare many drugs (11).

Nanoparticles-based compounds usually have better or different qualities as antibacterial than the bulk material of the same element. This could be attributed to high size to volume ratio which found in nanomaterials. In the case of silver nanoparticles based compounds the antibacterial effect is greatly enhanced due to tiny size that causes immense surface area relative to volume. Therefore very small amounts of silver Nanoparticles-based compounds can lend antimicrobial effects to hundreds of square meters of its host material (7).

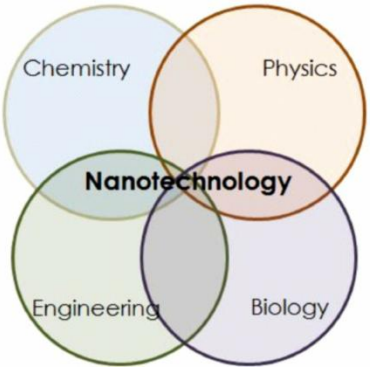

Figure 1 : Science of Nanotechnology

C. Cryptococcus neoformans:

Cryptococcus neoformans is a fungus that lives in the environment throughout the world. that can live in both plants and animals. People can become infected with C. neoformans after breathing in the microscopic fungus, although most people who are exposed to the fungus never get sick from it. C. neoformans infections are extremely rare in people who are otherwise healthy; most cases occur in people who have weakened immune systems, particularly those who have advanced HIV/AIDS.

Infection with C. neoformans is termed cryptococcosis. Most infections with C. neoformans occur in the lungs. Infection with the fungus Cryptococcus is called cryptococcosis. Cryptococcosis usually affects the lungs or the central nervous system (the brain and spinal cord), but it can also affect other parts of the body. Brain infections due to the fungus Cryptococcus are called cryptococcal meningitis.

Material and method:

A.Materials and Devices:

1)Centrifuge. 2) oven 3) Sensitive Balance 4) Micropipettes.
5) Incubator 6) Autoclave 7) Laminar flow cabinet .

B.Chemical Materials :

1) $AgNO_3$ (99.5%) 2) $C_6H_5O_7Na_3$ (98%) 3) Sabauroud Dextrose Agar

C. Method:

Preparation Nanosilver particals via Sol-Gel method through preparation of Aqueous Solution of $AgNO_3$ via dissolving (2.5 g) of silver nitrate in (800 ml) of deionizewater, solution (1). Then ,preparation solution (2) from dissolving (4.56 g) of ($C_6H_5O_7Na_3$) in 125 ml deionize water .After preparation solution 1 and 2 , put solution 1 on magnetic stirrers with run heat element till boiling then add solution 2 drop by drop with motion and heat element together, addition process of drops is takes 30 mn. After completion of the add-on process note that the color of the solution is changed. In the beginning it is light yellow and after period is a pale then , it turns yellow and turn brown then, removed from the heating element and left to cool down at room temperature.

Then, The centrifugal machine was used in 10,000 rpm (10 min), used test tube for filtteration , then the material was collected, the distilled water was added and the centrifuge was again placed. Repeat this process twice to wash the output to get rid of all Non-reactive ions, And then put the product in the oven at a temperature (40) for drying one day after that removed from the oven and collected the material as powder gray . Nanoparticals that studied their characterization like XRD and AFM in previous study .

D. Test of biological activity with Nanosilver :

Test of biological activity with nanosilver against five isolated samples of *Cryptococus neoformans* that is isolated from patients, that diagnosed in central department-Karbala/ Karbala city,and were grown on SDA (Sabouraud dextrose agar media at 37 C° for 3 days, prepared 5 tubes of nutrient broth, sterilizes by Autoglave, cooled , then inoculated growth formed on SDA media. And labeled of sampled tubes (1-5) sample , and incubate at 37C° for 3 h. and prepared 1000ml of SDA media in conical flask, sterilized by Autoclave, and pour 50 plates, then left to cool down and solidifies. Then, made three holes in each SDA plate on equal distances.then prepared (0.125mg/ml, 0.25mg/ml, 0.50 mg/ml, 5mg/ml, 10mg/ml, 15 mg/ml, 20 mg/ml, 25 mg/ml and control concentration 0.0mg/ml)from nanosilver particals solution that prepared from dissolve 100 mg. of nanparticales in 4 ml of Dimethyl SolfOxid (DMSO-biosolve) with vortex ,then , filled the 10 plates holes with 40 µg of the 10 prepared concentrations, placed into refrigerator at 4° for 1 h. to ensure well spread of nanosilver solution, then placed 50 µg from formed growth on media (tube no. 1) into SDA plates and spread it by L shape spreader, then incubate at 37 C° for 2 days. Accomplishing the previous steps on the other samples and other plates (each sample with 10 con. And control) .All previous steps are take place under complete sterilization conditions.Finally measured of inhibition zone and the recorded of results.

Table 1 :Concentration of Nanosilver particals solution.

Tube No.	5-FC-Zn-LH solution(stock)ml	VO. Of DMSO (ml)	Total conc.(mg/ml)
1	0.0	1	0.0
2	0.005	0.995	0.125
3	0.01	0.99	0.25
4	0.02	0.98	0.50
5	0.04	0.96	1
6	0.2	0.8	5
7	0.4	0.6	10
8	0.6	0.4	15
9	0.8	0.2	20
10	1	0	25

RESULTS

In this study, the biological activity of silver nanoparticles as an antimicrobial agent was studied and demonstrated that the inhibition zone is increased according to concentration of nanosilver particles solution.

Results gained from tables (2) showed that *Cryptococus neoformans* was sensetive to the Nanosilver particals . Minimum Inhibitory concentration(MIC) were 10 mg /ml for all *C. neoformans isolate*, (Fig.3).

Results of statistical analysis indicated of the table (2) there is significant differences ($p<0.01$) among isolates of samples which studied from *cryptococus neoformans*, where, isolate N0. 2 was most sensetive with nanosilver particals and gave greatest of inhibition zone for all concentration studied within Nanoparticals , mean of inhibition diameter 12.020 mm, followed (4,3,5) without significant differences ($P>0.01$) among these three isolates, whereas, the isolation (NO.1) was lowest sensetive compare with the other isolates , the mean of inhibition diameter for isolates (NO.1) is 6.030 mm. Also, results of statistical analysis showed in table (2), that no significant differences ($P > 0.01$) for concentrations (1000, 500, 250 and 125) µg/ml compare with the control and among same this concentrations,where, this concentration not appear inhibition level for all isolates. Whereas, increase in mean of inhibition diameter significante ($P<0.01$) for concentrations (5, 10, 15,20,25) mg. Of nanosilver particals. In general increasing of nanosilver particals concentration there is increase mean of inhibition diameter for all isolates.

figure 2 *: Cryptococcus neoformans*

figure 3 : (MIC) of nanosilver against *Cryptococcus neoformans*(10 mg/ml)

Table 2 : Inhibition activity of nanosilver against *C. neoformans*

Con. / Isolate.	Cont.	125µg	250µg	500µg	1 mg	5 mg	10 mg	15mg	20mg	25mg	Mean
1	0	0	0	0	0	8.32	10	12	14.32	15.66	6.030
2	0	0	0	0	0	19.32	20	24	26.32	26.66	12.020
3	0	0	0	0	0	16.66	19.32	21.32	24	26.32	10.760
4	0	0	0	0	0	10.65	20	23.32	25	28.32	10.832
5	0	0	0	0	0	13	15.32	17.32	27.32	29.32	10.231
Mean	0	0	0	0	0	13.5	17.92	19.58	23.38	25.65	

The factory	Isolates	concentration	Interference
$LSD_{0.01}$	0.368	0.521	1.166

DISCUSSION

Nanoparticals, have recently obtain much attention because of their unique physical and chemical properties. smart nanoparticals properties of nanosilver offer a new scope for developing materiala at nanoscal or the so-called nanomaterials . nanosilver attracting much attention in antimicrobial due to their biocompatibility.

In this study, preparation of nanosilver particals via Sol- gel method from mixing $AgNo_3$ solution that prepared from dissolve 2.5 g. of $AgNo_3$ in 800 ml of deionized water with $C_6H_5O_7Na_3$ solution that have been dissolve 4.56 g. of $C_6H_5O_7Na_3$ in 125 ml of deionized water for the formation of nanoparticles

Former studies using silver nitrate has been carried out using coprecipitation method to gain nanosilver particals .(Tagree et al., 2015). Characterization of Nanosilver in previous study using X-Ray Differactogram , and AFM images that showed in prior study , where, AFM of the nanopracticales compelementary of the XRD results and provide further evidenece for the nano properties (Ghassan et al.,2013).

The effect of silver nanoparticles (AgNPs) on the antibacterial activity of four different antibiotics (amoxicillin, chloramphenicol, and penicillin) against Gram-positive and Gram-negative isolates (Mah, 2012). Also, effect of nanosilver practicle as antifungal on *candida albicans* (Hamss et al., 2013).

Results gained from test of silver nanoparticles within *Cryptococus neoformans*, showed that *C.neoformans* was sensitive to the silver nanoparticles .

RECOMMENDATION

Based on this study , put the following recommendation, studing the effect of nanosliver combine with anather antibiotics . and testing the activity of the nanopracticles with the store time is important to show the effect of to fix the time of expiration.