The effects of *Fusarium graminarum* silver nanoparticles on *leishmania tropica*

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**Abstract.** The present study aims to evaluate the *in vitro* antileishmanial activities of biosynthesis silver nanoparticles from *Fusarium graminarum* and to compare its efficacy with a pentostam drug against *Leishmania Tropica*. This nanoparticles (20-40-80-100 μg/mL) was evaluated *in vitro* against promastigote and intracellular amastigote forms of *L. tropica* also measured the viability by use MTTassy. Its significantly (*P* < 0.05) inhibited the growth rate of promastigote the results showed decrease in number of parasite after 24hr was \((1816.6, 1300, 541.6, \times 10^4) \text{ cell/ml} \) respectively, compared with pentostam and control was \((1558.3 \text{ and } 2441.6, 308,3\times 10^4) \text{ cell/ml} \) respectively. whereas after 48hr the number of parasite became \((1583.3,1166.6,450, 225\times 10^4) \text{ cell/ml} \) respectively compared with pentostam and control was \((1066.6 \text{ and } 3166.6\times 10^4) \text{ cell/ml} \) respectively, also observed (100 μg/mL) more effects on multiplication ofamastigote after 24hr become only 1.6 parasite compared with pentostam and control was \((3.3,7 \text{ parasites}) \). The results of viability also revealed that the nanoparticles Cytotoxicity effect dependent on concentration and reduce to reach (23% ). Obtained findings also provide the scientific evidences that fungi could be used in the biosynthesis nanoparticles and treatment of CL.

**Keyword:** leishmania tropica, nanoparticles, pentostam, promasigote.

**Introduction**

Cutaneous leishmaniasis is caused by different species of *leishmania*, it is endemic in 88 countries like Iraq, Syria, India, Iran, Sudan, Bangladesh and Brazil (1). It spread by sand fly bites, its intracellular protozoa (2). Cutaneous leishmaniasis is difficult to treat and there is increasing resistance against the current drugs, so there is an urgent need for new therapy and vaccine against leishmaniasis (3). Silver nanoparticles are widely used as catalysts for methanoloxidation to formaldehyde and carbon monoxide to carbon dioxide, Primarily the reduction of Ag+ ions leads to the formation of silver atoms (Ag) which is followed by accumulation into oligomeric groups. These groups ultimately lead
to the formation of colloidal Ag particle. (4, 5). Silver nanoparticles have a wide antibacterial effect on a range of Gram-positive and Gram-negative bacteria as well as antibiotic-resistant bacteria strains(6).also have actual antifungal agent against a wide spectrum of common fungi.( 7)and antiviral agent against HIV-1, hepatitis B virus (HBV), herpes simplex virus type 1, respiratory syncytial virus, and monkey pox virus (8). *Fusarium graminearum* is a big genus of filamentous fungi, part of a collection often mentioned to as hyphomycetes, widely spread in soil and associated with plants, fungi can produce greater quantities of nanoparticles due to they can produce a high total of mycelia mat and secrete large totals of proteins which directly translate to higher productivity of nanoparticles (9). The aims of the present study of biosynthesis metal oxide nanoparticles from *Fusarium graminearum* and study the effects on *leishmania tropica* invitro.

**Materials and Methods**

**Silver nanoparticles (AgNPs) preparation:**

*Fusarium graminearum*

*Fusarium graminearum* have got from the Department of Biology/ Collage of Science Mustainsiriyah university in Baghdad / Iraq, isolated from the decayed banana fruit. Then grown in Potato dextrose Agar medium (PDA) for use in the present study to biosynthesize of silver nitrate Ag (NO3) 2 nanoparticles.

**Production of biomass**

The of *Fusarium graminearum* mycelia were inoculated in 250 ml flasks, each flask containing 100 ml of PDB medium, then Incubated at 25 ± 2°C for 5 days. The pH of the media was adjusted to 5.6.(10)

**Nanoparticle synthesis**

Fungi mycelia were collected by filtration through Whatman filter paper No. 42 then washed thrice with deionized water to eliminate the traces of the medium on fungal biomass. The mycelia pellet was re-suspended in 100 ml distilled water, then incubated at 25°C for 2days. Once more, mycelia were collected by filtration through Whatman filter paper No. 42. Then, cells filtrate were divided two parts, first one treated with 1mM silver nitrate solution and incubated at room temperature, which change color to brown consider as Positive control, while the second part left without the addition of AgNO3 to the cells filtrate without change in color consider as negative control. (11).

**Characterization of silver nanoparticles**

The biosynthesis were confirmed by UV–VIS Spectroscopic Analysis used for investigating the features of nanomaterials involved aggregation state, concentration, size and even bio-conjugation when the absorption shapes of nanomaterials are distinct by passing light through a sample and the transmittance of light by a sample is measured.

- X-ray Diffraction Analysis (X-RD) used for determining the crystallite size of silver nanoparticles in solution
- Atomic absorption spectroscopy flame (AASF) examines the concentration of elements in a liquid sample depend on energy absorbed from confident wavelengths of light.

The sample was examined in the collage of education Ibn Al-Haytham /University of Baghdad.

Parasite strain and culture

*Leishmania tropica* the strain was obtained from biotechnology center/ AL- Naharin University, it was cultured and maintained by serial passage in NNN media each 8 days and incubated at 26°C.

-Antipromastigote effect

Added 1ml from promastigote culture contain \(2 \times 10^6 \text{ parasite/ml}\) in plain tubes that contain 1ml from RPMI 1640 medium as triplicate , then added 20, 40, 80 and 100 µg/ml of *Fusarium* silver nanoparticles (FSNPs). In a negative control group, promastigotes left without added nanoparticles while the treatment group added Pentostam 100 µg/ml , after 24 and 48 h of incubation the multiplication of the promastigotes was measured by counting the number of parasites by hemocytometer chamber.

-Cytotoxic effect of FSNPs on *L.tropica* promastigote.

Promastigote ,harvested in the exponential growth phase, resuspended in RPMI medium in \(1 \times 10^6 \text{ cells/ml}\) were put in 96-well culture plate. then FSNPs was added in triplicate at 20, 40, 80 and 100 µg/ml. The plate was incubated at 27°C for 24h, parasite viability was evaluated by MTT assay. The method described by (12). Finally , absorbance was measured by an ELISA reader at at wavelength 590 nm

The percentage of viability was calculated from OD readings according to the following formula: (13)

\[
\text{Viable cells} = \frac{\text{absorbance of treated cells}}{\text{absorbance of control cells}} \times 100
\]

-Preparation of murine macrophages

Murine macrophage cells were collected from BALB\'c mice (4-7 weeks) by injecting intraperitoneal 3-5ml of cold RPMI-1640 medium, then aspirated fluid contain macrophages, washed and resuspended in RPMI-1640 medium. (12).

Effects of FSNPs on intramacrophage amastigote.

Added 200µL of the murine macrophages(\(10^6 \text{ cells/mL}\)) in 96-well culture plate and promastigotes were put in each well with RPMI-1640 medium and incubated at 37°C for 24h,then free parasites were removed by washing with RPMI-1640 medium, and the infected macrophages treated with FSNPs 20, 40, 80 and 100 µg/ml or 50µL from Pentostam or macrophage alone as a control, incubated for 28h, finally make slide for each concentration, fixed with methanol and stained by Giemsa, and studied by light microscope, then the infected macrophages were measured by counting 100macrophage. All experiments were carried out in triplicate.

Statistical analysis

The Statistical analysis System- SAS (2012) program was used to affect different factors in study parameters. Slightest important difference –LSD test (ANOVA) was used to significant compare between earnings in this study.
Results and discussion

The reaction between *Fusarium graminearum* biomass and Ag(NO3) led to change the color of the mixture to blackish brown this is indication of silver nanoparticles formation and due to the excitation of surface Plasmon vibrations in the silver nanoparticles. Control no change in color under the same condition. These results corresponding with (14). then using the software of the AFM for Determine nanoparticles (AgNPs) sizes and surface morphology the average diameter is 94 nm figure [1]. while the average roughness (Ra) is 9.33 nm and Root mean square (Sq) is 11.6 nm figure [2]. AFM topology is very helpful in revealing the exact size and shape of silver nanoparticles (15).

The XRD technique is used to identify and characterize compounds the diffraction peaks at 38.05°, 44.22°, 64.32° and 77.31° were correspondent to the (111, 200, 220 and 311) the faces of the face centered crystal cube structure, therefore the average crystallite size was 28.225 nm. figure [3] similar results have been reported by Gholami et al. (16) when extracted nanoparticles from *Fusarium oxysporum* fungi. To confirm the presence of AgNPs by measuring the absorbance of the bio-reduced solution at wavelengths between 300 and 800 nm, which showed a peak absorption at 420 nm, figure [4], another study showed that the absorption peak is about 420 nm, which is specific to AgNPs (17). Singh et al. (18) reported that the biosynthesis of AgNPs by using endophytic fungus *Fusarium* spp. Found the maximum surface Plasmon resonance peak at 420 nm

![Granularity Cumulation Distribution Chart](image)

**Figure (1):** Granularity volume distribution chart of Fusarium silver nanoparticles
Figure (2): AFM images of *Fusarium* silver nanoparticles

Figure (3): X-Ray pattern of *Fusarium* silver nanoparticles
-Antipromastigote effect

The effect of different concentration (20, 40, 80, 100) µg/ml of FSNPs on promastigote against the culture contain promastigote after 24 and 48hr incubation and compared it with pentostam revealed that the FSNPs give highly effect on a number of parasites, the results showed decrease in the number of parasites after 24hr was (1816.6, 1300.541.6, ×10^4) cell/ml respectively, compared with pentostam and control was (1558.3 and 2441.6, 308.3×10^4) cell/ml respectively. Whereas after 48hr the number of parasites became (1583.3, 1166.6, 450, 225×10^4) cell/ml respectively, compared with pentostam and control was (1066.6 and 3166.6×10^4) cell/ml respectively. Table[1].

Enad and Zghair (19) examined that carbon nanotubes (CNTs) antiparasitic activity on the Iraqi strain of L. donovani promastigots. Other nanoparticles such as metal derived ZnO NPs have revealed that these nanoparticles have shown a strong tendency to produce ROS in Pathogens and developed oxidative stress, which cause membrane damage by electrostatic binding (20). Nadhmanet al. (21) have previously used PEGylated (polyethylene glycol) silver doped zinc oxide nanoparticles as novel photosensitizers for photodynamic cure against Leishmania, the nanoparticles have caused the increase permeability of the cell membrane, resulting in the death of the parasites.

**Table 1.** Effect of FSNPs and pentostam in number of parasites (1×10^4)

| Time | Control | Pentostam | 100 µg/ml | 80 µg/ml | 40 µg/ml | 20 µg/ml | LSD value |
|------|---------|-----------|-----------|----------|----------|----------|-----------|
| 24hr | 2441.67 ± 36.32 | 1558.33 ± 36.32 | 308.33 ± 16.67 | 541.67 ± 22.05 | 1300.00 ± 14.43 | 1816.67 ± 14.43 | 217.39* |
| 48hr | 3166.67 ± 110.24 | 1066.67 ± 30.04 | 225.00 ± 14.43 | 450.00 ± 14.43 | 1166.67 ± 22.04 | 1583.33 ± 22.04 | 196.42 |
| LSD value | 322.26 * | 130.88 * | 61.21 * | 73.16 * | 73.16 * | 142.63 * | --- |

* P<0.05).
Cytotoxic Effect of FSNPs on L.tropica promastigote

The effect of different concentrations (20, 40, 80, 100) µg/ml of FSNPs on promastigote after 24 was evaluated. The viability of promastigote after 24hr showed significant (p<0.05) differences between all the concentrations and Pentostam also the decrease viability with increased concentrations (23%, 46%, 57%, 68%) respectively compared with pentostam was 65%. Table (2). Ghadi et al. (10) reported that the percentage of viability of promastigote after 24hr of after exposure to Fusarium AgNPs (2.5, 2, 1.5, 1 µg/ml) were (39, 42, 44 and 49%) respectively, and also after 48 hours of exposure to Fusarium AgNPs and pentostam drug the results showed the percentage of viability was decreased and became (23, 37, 40 and 43%) respectively. Al-Saeedi and Saheb (22) reported that Ag NPs consider as anti-leishmanial effects by inhibiting the promastigotes multiplying and metabolic activity.

Zahir et al. (23) showed that, the AgNPs enhanced a effective dose dependent anti-proliferative effect on Leishmania infantum promastigotes, the extracellular Leishmania stage can inhibit trypanothione reductase (TR) at nanomolar concentrations.

**Table 2. Effect of AgNPs and pentostam in Viability( %) of promastigote**

| Groups             | Viability( %) of promastigote |
|--------------------|-------------------------------|
| AgNPs20 µg/ml      | 68                            |
| AgNPs40 µg/ml      | 57                            |
| AgNPs80 µg/ml      | 46                            |
| AgNPs100 µg/ml     | 23                            |
| Pentostam (100 mg/ml) | 65                           |
| LSD value         | 9.321 *                       |

Anti-leishmanial effects of Fusarium AgNPs on amastigote

The effect of Fusarium AgNPs and pentostam in amastigote multiplication was evaluated by calculating the number of parasite within infected phagocytic cells. It was observed that after 24 hours of incubation, Fusarium AgNPs in different concentrations and pentostam led to reduce the division of amastigotes within infected phagocytic cells. The mean number of amastigotes within each infected phagocytic cell for both Fusarium AgNPs concentrations, pentostam and control was (1.22, 2.11 and 4.9) respectively, Table [3]. There was significant difference between Fusarium AgNPs concentrations, pentostam and control. The results showed that parasites lose their infection capabilities following exposure to nanoparticles, therefore these results are similar to Ullah, (24) who reported that the Leishmania parasite lose their ability to infect the macrophage cells after exposure to the AgNPs. Noble metal nanoparticles are induce producing ROS, which can destroy pathogenic microbes through a process called respiratory explosion mechanism. It has been mentioned earlier that Leishmania is high Sensitive to these oxygen species and drug, which could produce ROS, will be an effective anti-leishmanial agents (25).
Table 3. Effect of AgNPs and AgNPs in a number of amastigotes/phagocytic cells

| Groups          | Mean number of amastigotes/phagocytic cells Mean ± SD |
|-----------------|------------------------------------------------------|
| AgNPs20 µg/ml   | 5.67 ± 0.33                                          |
| AgNPs40 µg/ml   | 5.00 ± 0.57                                          |
| AgNPs80 µg/ml   | 3.67 ± 0.33                                          |
| AgNPs100 µg/ml  | 1.67 ± 0.33                                          |
| Pentostam (100 mg/ml) | 3.33 ± 0.33                               |
| Control         | 7.00 ± 0.57                                          |
| LSD value       | 1.326 *                                              |

* (P<0.05).

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