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Effect of *Fusarium graminarum* silver nanoparticles on liver tissue of infected mice of visceral leishmaniasis

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Abstract

Leishmaniasis remains one of the fatal diseases worldwide, and the conventional antileishmanial therapies are toxic and most are expensive. Biological silver nanoparticles possess broad-spectrum antimicrobial activities and could be a future alternative to current antimicrobial agents. In the present study an approach was made tobiosynthesis silver nanoparticles using a *Fusarium graminarum* fungus. These nanoparticles are described to confirm the shape, size .The efficiency of *Fusarium* silver nanoparticles against *Leishmania donovani* compared with pentostam drug in liveron the mice**,** it was observed that *leishmania* parasites after the third week led to the occurrence infiltration of lymphocytes, activation of kupffer cells and multiple microgranuloma in paranchyma.The dosage of 21day infected mice with AgNPs led to the few infiltration of lymphocytes with the return of the tissue to almost normal.While the treated of mice infected with the pentostam drug for 21 days led to a slight repair of liver tissue with the occurrence of hydropic degeneration and mild haemorrahage.As for treatingmice with pentostam drug and *Fusarium* AgNPs together, noticed hydropic degeneration and necrosis occurred.

Keyword: *Leishmania donovani*, silver nanoparticles, *Fusarium graminarum*

INTRODUCTION

Leishmaniasis is one of the vector-borne diseases caused by obligate protozoan parasites of the genus *Leishmania*, they are transmitted by different species of sand flies belong the genus of *Phlebotomine* as extracellular flagellated promastigotes that replicate as an intracellular parasite (aflagellate amastigotes) in mononuclear cells of mammalian hosts (1). Visceral leishmaniasis is considered as the second cause of mortality and the fourth cause of morbidity after malaria, schistosomiasis and Africantrypanosomiasis (2). Visceral leishmaniasis affects more than 100 million people worldwide, with 500,000 new cases occur and more than 50,000 deathseachyear.

Overof90%casereportedfrom India, Bangladesh, Nepal, Su dan and Brazil (3). The Clinical symptoms of visceral leishmaniasis infection include a grossly enlarged abdomen due to associated hepatosplenomegaly and splenomegaly, and general symptoms such as irregular fever, loss of appetite and weight, malaise, chills, wasting, pallor of mucous membranes and hypergammaglobulinemia (4). Pentavalent antimonials are a group of compounds used for the treatment of leishmaniasis. The compounds currently available for clinical use are sodium stibogluconate and meglumine antimonate. In systemic therapy of leishmaniasis these drugs are used alone or in combination with other compounds. The current drugs is not so much suitable due to resistance reported, high toxicity, various side effects and so forth. So, new therapeutic antileishmanial strategies are urgently required (5).The role of cytokines such as IFN-γ is to activate macrophages and enhance the microbicidal activity of these cells to kill intracellular pathogens through the generation of reactive oxygen species and reactive nitrogen species.IL-10 promotes intracellular infection, including human visceral leishmaniasis, by disabling Th1 cell type responses and/or deactivating parasitized tissue macrophages (6).Nanotechnology continues to attract significant attention due to its impact on many currently important areas such as energy, medicine, electronics and the aerospace industry. It's that possess one or more dimensions of the order of 100 nm or less continue to attract significant attention due to their unique properties in the realms of chemistry, optics, electronics and magnetism (7). The use of eukaryotic organisms such as fungi, *Fusarium graminarum* and other species offers considerable promise for large-scale metal nanoparticle production since the enzymes that are secreted by the fungi represent an essential ingredient for the biosynthesis of metal silver nanoparticles has attracted high interest due to their unique and excellent properties in addition to its therapeutic potential for the treatment of a variety of diseases that includes retinal neovascularization and acquired immunodeficiency syndrome due to human immunodeficiency virus (8).

MATERIALS AND METHODS

Silvernanoparticles (AgNPs) preparation:

The mycelia of *F. graminearum* were inoculated in 250mL erlenmeyer flasks, each flask containing 100mL of potato dextrose broth (PDB) medium, then incubated at 25 ± 2°C for 5 days. Later, mycelia were harvested by filtration through Whatman filter paper No. 42 and washed thrice with sterilized distilled water to remove the traces of the medium on fungal biomass. The washed mycelia were resuspended in 100mL sterilized distilled water, then incubated at 25°C for 24hours. Again, mycelia were harvested 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 $AgNO_3$ to the cells filtrate without change in color consider as negative control.

Characterizationof nanoparticles

The detection of silver nanoparticles (AgNPs) was primarily carried out by visual observation of color change of the fungal filtrate after adding silver nitrate. The appearance of dark brown color. The exact configuration of the, size, concentration, morphology of crystals, aggregation state and even bioconjugation and was measuredby using particle the following

techniques:Atomic force microscopy (AFM)(AA-3000, Angstrom, USA), X-Ray diffraction (XRD)(Shemadzu, Japan), Ultraviolet-visible spectroscopy (UV-VIS)(Shemadzu, Japan).

Parasite strain and culture

Leishmania donovani was isolated from the bone marrow of an infected child , 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.

***Leishmania* antigen preparation**

One milliliter of promastigote culture in stationary phase washed three times with phosphate buffered saline by centrifuge 4000 rpm in 15 minutes then adjusted to concentration 1×10^7 parasite\ml.

Animals

Ninety six Male *albino* mice aged between 8-12 weeks, weighing 20-28 gm was obtained from National Center for Drug Control and Research, housed under standard condition in animal house in the biology department in College of Science/AL-Mustansrya university .

Seventy eight mice were infected with 1×10^7 parasite/ml *L. donovani* promastigotes by injectionintraperitonial (9). Then the groups were inoculated as a follow:

- Group 1 : Inoculated orally by stomach tube (0.1ml/day) normal saline considers as control positive group.
- Group 2 : Inoculated orally by stomach tube AgNPs (0.1ml/day) for 21 days considers as an AgNPs treatment group.
- Group 3 : Injected with 0.01ml (0.041mg/kg)from Pentostam drug by intramuscular each day for 21 days considers as Pentostam treatment group.
- Group 4 : Inoculated orally by stomach tube AgNPs (0.1ml/day) and injected with 0.01ml (0.041mg/kg)from Pentostam drug by intramuscular for 21 days consider as AgNPs and Pentostam treatment group.
- Group 5: Inoculated orally by stomach tube (0.1ml/day) normal saline considers asnegative control without infecting by *L. donovani* parasite.

Histopathological study

Livers were removed from the sacrificed mice and fixed in 10% formalin, processed and staining with hematoxylin and eosin then examined under the light microscope to study histopathological changes.

Statistical analysis

The Statistical Analysis System- SAS (2012) program was used to effect of different factors in study parameters. Least significant difference –LSD test (ANOVA) was used to significant compare between means in this study, (10).

RESULTS AND DISCUSSION

Detection of the existence of *Fusarium* AgNPs

The synthesis of silver particles by using *Fusarium graminarum* fungiwas examined. After adding of silver nitrate ($AgNO_3$) to filtered cell, the color of the mixture changed from colorless to blackish brown compared with negative control remain clear without color (colorless) fig. (1) , the changes in color which confirms the reduction of $AgNO_3$ by *F. graminarum* indicated the presence of AgNPs.

The synthesis of silver particles by using *Fusarium graminarum* was observed during change the color of the mixture from colorless to blackish brown, the color change confirmed the formation of nanoparticles, these results corresponding with (11).

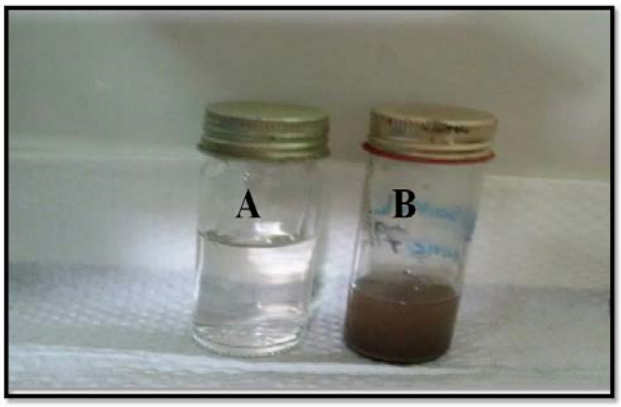
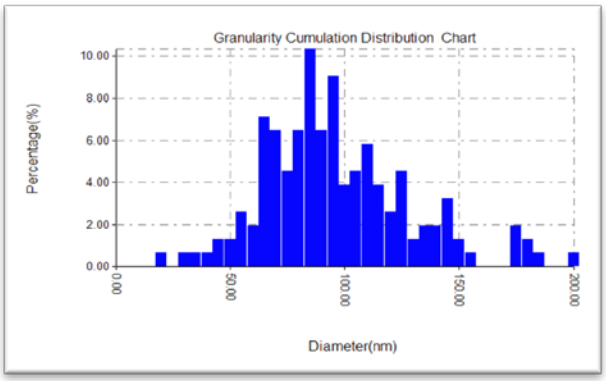


Figure (1): Color change reaction ofsilver nanoparticles: (A) negative control showed colorless, (B) Fifiltrate of *F. graminearum* cellscolored blackish brown after adding $Ag(NO_3)_2$.

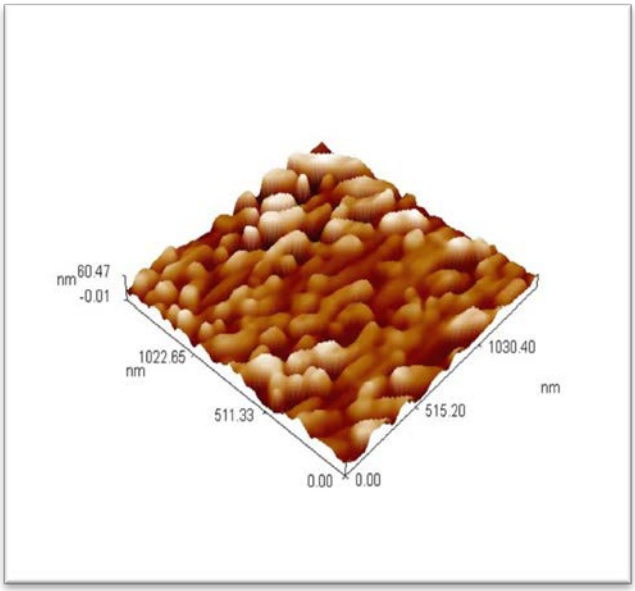
Morphology of*Fusarium*silver nanoparticles by atomic forcemicroscopy

Determine *Fusarium* silver nanoparticles (AgNPs) sizes and surface morphology were measured ,using the software of the AFM , the images of AFM for *Fusarium* AgNPs in fig. (2) represents particle size distribution, where average diameter is 94 nm. While in fig.(3A,B) is AFM picture in three dimensions (3D) and two dimensions (2D), it explains structural shape for grains,found that the average roughness (Ra) is 9.33 nm and Root mean square (Sq) is 11.6 nm.

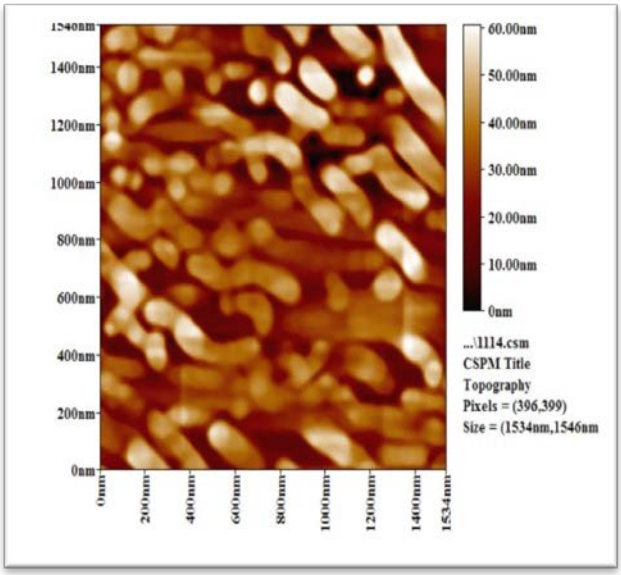
AFM is a very good technique for measuring surface morphology and fine structure of nanoparticles (12). (13)Vijayan *etal.* (2016) observed that AFM topology is very helpful in revealing the exact size and shape of silver nanoparticles.



Figure(2):Granularity volume distribution chart of silver nanoparticles produced by *F. graminurum*



A



B

Figure (3): AFM images of silver nanoparticles produced by *F. graminurum* (A) three dimensions 3D and (B) two dimensions 2D

Characterization of *Fusarium* AgNPs by X-RayDiffraction

XRD technique is used to identify and characterize compounds based on X-ray diffraction pattern. A typical XRD pattern of *Fusarium* AgNPs as shown in fig.(6), the diffraction peaks at 38.05°, 44.22°, 64.32 and 77.31° were correspond to the (111), (200), (220) and (311) facets of the face centered cubic crystal structure, therefore the average crystallite size was 28.225 nm.

In this study, the results are near to results of (14)Shafiq *et al*., (2016)showed that, the XRD diffraction measured in Ag-NPs resulted in four intense peaks and this further confirms that the Ag-NPs formed in the extracellular filtrate are present in the form silver nanocrystals.

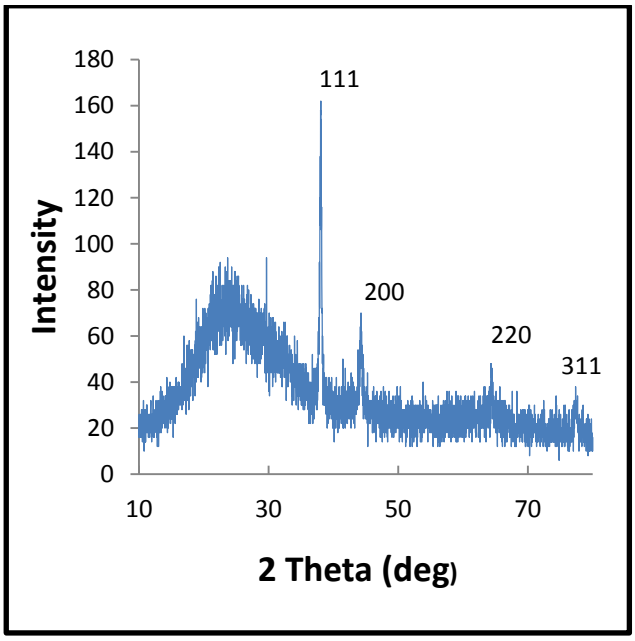


Figure (6): X-Ray pattern of silver nanoparticles produced by *F. graminurum*.

Optical properties of *Fusarium*AgNPs of UV–Visible Spectral:

This technique confirms the presence of *Fusarium* AgNPs by measuring the absorbance of the bioreduced solution at wavelengths between 300 and 800 nm. Extinction spectroscopy of ultraviolet (UV) and visible (Vis) light (UV–Vis spectrum) allows confirming the presence of *Fusarium* AgNPs because of the characteristic plasmon resonance, which showed an absorbance peak at 420 nm, fig (7).The results corroborate those of previous studies such as (15)Birla *et al.*, (2013) showed that absorbance peak around 420 nm, which is specific for the SNPs.

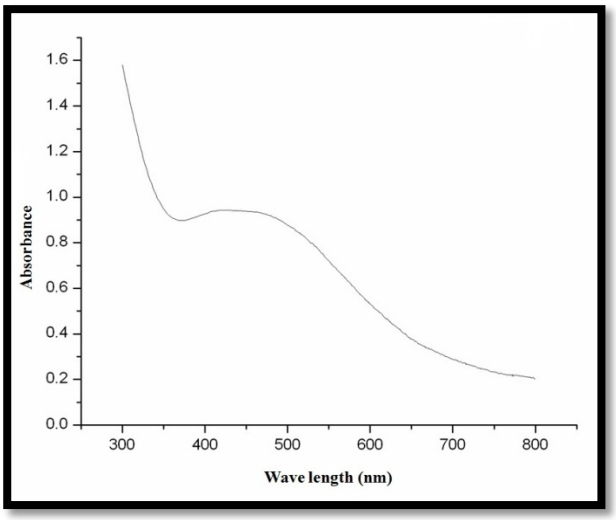


Figure (7):UV-Visible spectroscopy of silver nanoparticles produced by *F. graminurum*.

Histopathological study of liver

After fourteen days of parasite infectionsix mice were sacrificed , remove the liver and prepared impression

smears on a slide , stained by Gamisa stain and examined under a microscope to confirm the presence of amastigotes inside a kuppfer cells, as shown in fig.(4). After first week post-infection, histological changes in the liver appeared in comparison to non-infected mice fig.(5). The hisological changes after the first week from infected parasite showsinfilteration of lymphocytes ,multiple microgranuloma in paranchyma and activation of kupffer cells fig.(6). Whileafter third week the changes have become more severe and shows infilteration of lymphocytes, multiple microgranuloma in paranchyma, haemorrhage and activation of kupffer cells fig.(7).While the liver section for mice treated by *Fusarium*AgNPs showed repair occurred in the paranchamel liver after one week with infiltration of lymphocytes and hydropic degeneration fig.(8), and after three weeks shows only the few infiltration of lymphocytes occurring fig.(9).

The liver section of mice treated with the pentostam drug after one week shows infiltration of lymphocytes, hydropic degeneration and proliferative of kupffer cells fig.(10). In this study the results showed a little hepatic changes occurred in the pentostam group after three weeks shows occurs repairmen of tissue with hydropic degeneration and mild hemorrahage fig.(11). But after treating with pentostam and *Fusarium*AgNPs, the histological liver change after one week shows an aggregation of lymphocytes, hydropic degeneration, active proliferative of kupffer cells and necrosis fig.(12). And after three week noticed hydropic degeneration and necrosisoccurred as shown fig. (13).

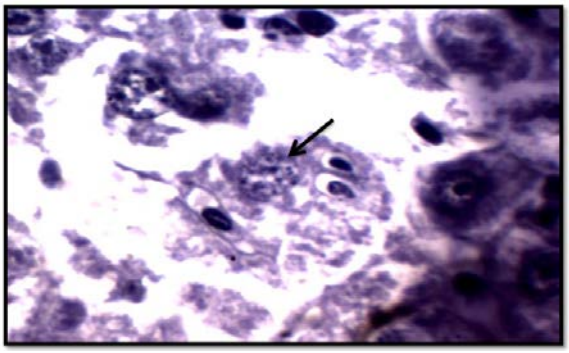
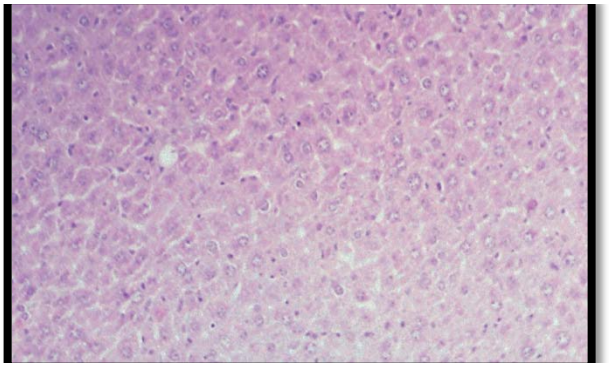
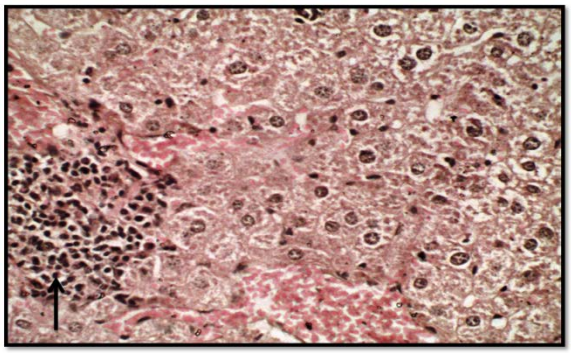


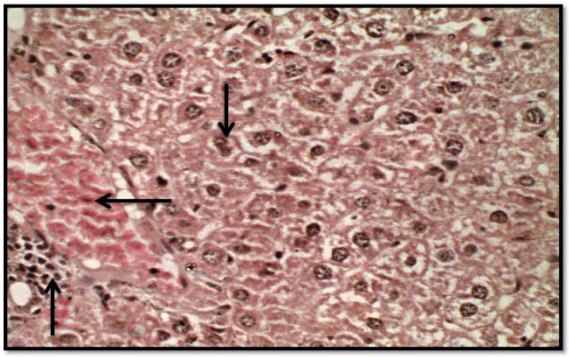
Figure (4): Liver smear showing *Leishmania donovani* parasites in a kuppfer cells from infected mice (Giemsa stain,100x).



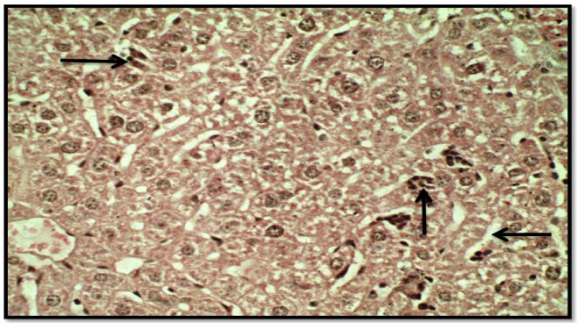
Figure(5): Liver section shows the normal liver structure for non-infected mice.(H&E.40X)



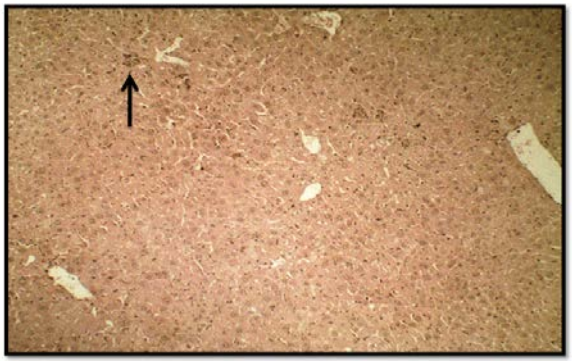
Figure(6): liver section of mice infected with *Leishmania donovani* after 7days shows infiltration of lymphocytes, multiple microgranuloma in paranchyma and activation of kupffer cells. (H&E.40X)



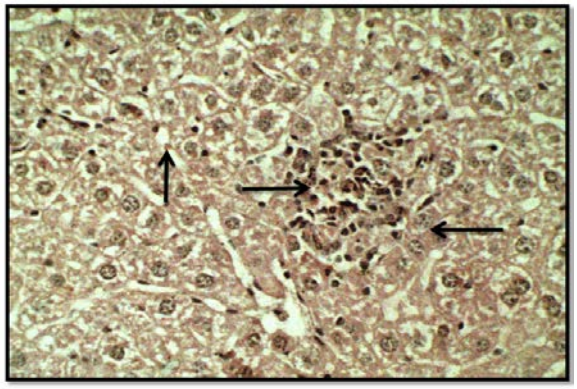
Figure(7): liver section of mice infected with *Leishmania donovani* after 21days shows infiltration of lymphocytes, hemorrhage and activation of kupffer cells . (H&E.40 X).



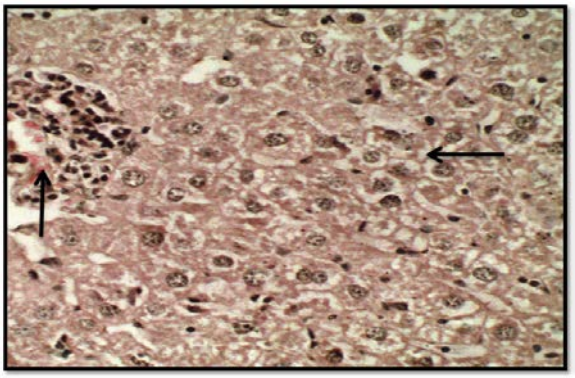
Figure(8):Liver section of mice treated with *F. graminarum* silver nanoparticles after one week shows apoptosis with infiltration of lymphocytes and simple dialation in sinuside sinuses (H&E.40 X).



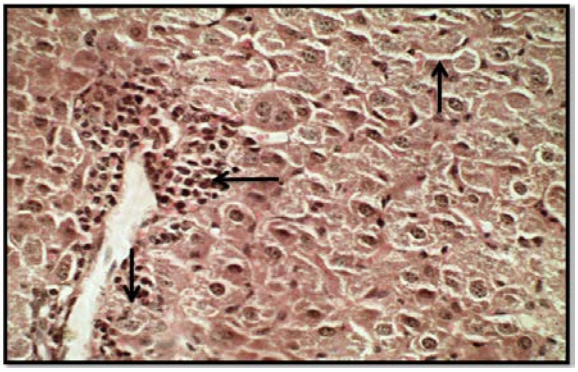
Figure(9):Liver section of mice treated with *F. graminarum* silver nanoparticlesafter three week shows occurring repairment with afew infiltration of lymphocytes (H&E.40 X).



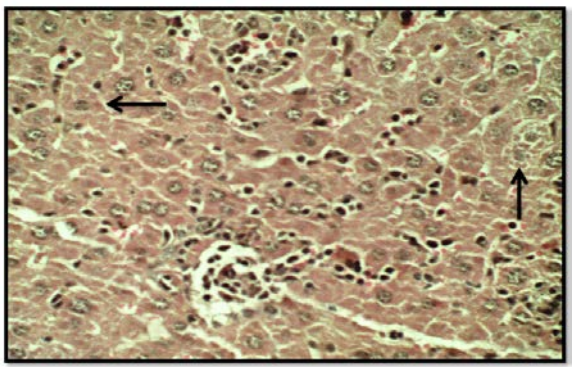
Figure(10): Liver section of mice treated with pentostam after one week shows infiltration of lymphocytes, hydropic degeneration and proliferative of kupffer cells (H&E.40 X).



Figure(11): Liver section of mice treated with pentostam after three weeks shows occurs repairment of tissue with hydropic degeneration and mild haemorrahage (H&E.40 X).



Figure(12): Liver section of mice treated with pentostam and *F. graminarum* silver nanoparticles after one week shows an aggregation of lymphocytes, hydropic degeneration, active proliferative of kupffer cells (H&E.40 X).



Figure(13): Liver section of mice treated with pentostam and *F. graminarum* silver nanoparticles after three weeks shows hydropic degeneration and necrosis (H&E.40 X).

In the present study, no similar reports were found and thus unable to provide comprehensive information on the overall effectiveness of the *Fusarium* AgNPs on the parasite in the liver, and effects on liver tissue. The liver is first of the primary target organs in VL. (16) Kaye *et al.* (2004) confirmed that in experimental mice of visceral leishmaniasis, infection in the liver was self-resolving within 2–3 months. This resolve of disease was linked with the development of granuloma formation facilitated by a Th1 immune response together in dogs and humans (17).An effective granuloma formation includes the expression of iNOS by macrophages, which is controlled by several pro-inflammatory Th1 cytokines, such as IFN-γ, TNF-α, IL-12, stimulating factor, granulocyte/macrophage colony- lymphotoxin, IL-2 as well as intact and functional natural killer cell and natural killer T cell (NKT) (18).Visceral leishmaniasis leads to hepatic dysfunction, such as increased serum concentrations of several liver-specific enzymes, coagulation defects and changes in the cholesterol biosynthesis (19).

In a previous study it was reported that the furthermost significant soluble factor for granuloma development and hepatic control of *Leishmania* infection is TNF, which plays a vital role in coordinating the assembly and maturing of granulomas. In the lack of TNF, parasite growth in the liver profits undamaged through the first weeks because of the completely absent granuloma formation. However, later in infection (6–8 weeks) there is an abrupt assembly of granulomas causing fast death due to fulminant hepatic necrosis (20),at third weeks showed infiltration of lymphocytes, multiple microgranuloma in paranchyma, haemorrhage and activation of kupffer cells, so various studies reported that after one week post-injection of mice with *Leishmania donovani* the kupffer cells harbor most parasites and innate capacity reduced to kill intracellular *leishmania* in the early stage, of infection due to evade immune system (active stage) thus, increases rapidly hepatic parasite burden, and restriction of liver parasite number, parallel the assembly of inflammation structure, known as granuloma characterized by parasitized kupffer cell surrounded by a mantle of lymphocyte during the first two weeks (21).When treating infected mice in the present study with *Fusarium*AgNPs it showed repair occurred in the paranchyamel liver after one week with infiltration of lymphocytes and hydropic degeneration, and after three weeks showed only the few infiltration of lymphocytes occurring, the results were consistent with (22) Mohammed (2017) who reported that the repaires caused by liver tissues are due to silver nanoparticles. Another reports showed in the liver, reduction of parasite burden of *L. donovani* infection one week post-treatment withArtemisinin-loaded poly lactic co-glycolic acid (ALPLGA) nanoparticles at high doses was comparable to the levels with the typical drug, amphotericin B (AmB) caused by the slight size of the nanoparticles that are simply taken up by the macrophage phagocytic system as proved by other drug transport systems against the *Leishmania* infection, hence increasing the therapeutic convenience of the drug to the amastigote infected macrophages, thereby clearing the parasites

(23).Nanoselenium could have decreased the amastigote rate in liver of mice during the treatment (24). On the other hand,the hepatotoxicity of activity pentavalent compound, in murine model of visceral leishmaniasis, after end day of treatment (21 days) included an increase swollen, and vacuolization, of hepatocyte, that could be attributed to alteration in water homeostasis that may be caused by free radical, during reduction pentavalent antimonial [Sb(V)] to the trivalent form [Sb(III)] (25).

CONCLUSION

Silver nanoparticles synthesis from *Fusarium graminarum* is safety, it can be considered as a new antileishmanial agent. *Fusarium* AgNPs less toxicity than pentostam drug in liver tissues.

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