Histological Changes Induced by the *Fusarium graminarum* Silver Nanoparticles in Some Organs of Male Albino Mice

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Abstract

The aim of this study was to investigate the effects on male albino mice of the histological change of Fusarium graminarum, silver nanoparticles (AgNPs), and the traces of these nanoparticles in the liver, and small intestinal. The experiment consisted of 40 mice divided into two groups, the first group of 20 mice was considered to be the control animals and the other group was three weeks of treatment with a dose of AgNPs (0.1ml / day). Microscopic examination of the liver showed the disturbed structure of the hepatic lobule, hepatocyte hypertrophy with severs infiltration of inflammatory cells, kupffer cell proliferation, coagulates, necrosis, and hydropic degeneration. In addition, the small intestinal section showed hydropic degeneration in epithelium cells of the mucosa and an increased number of inflammatory cells also, the proliferation of goblet cells in epithelium cells of the intestinal mucosa and sloughing of necrotic villi into the intestinal lumen.

***Keywords**: Bionanotechnology, Fusarium graminarum, AgNPs ,liver,small intestinal*

Introduction

Silver nanoparticles (AgNPs) have unique properties that help in molecular diagnostics, in therapies, as well as in devices that are used in several medical procedures. The major methods used for AgNPs synthesis are the physical and chemical methods. The problem with the chemical and physical methods is that the synthesis is expensive and can also have toxic substances absorbed onto them [(1)]. AgNPs are presently the most heavily used, being incorporated for their antimicrobial characters in pillows, food storage containers, clothing up the holster and even toys for children [(2)]. The term "Myconanotechnology" for the research carried out on the synthesis of nanoparticles(NPs) by the fungal system. It is the interface between nanotechnology and mycology. Several fungi have the capability to produce various NPs like silver, cadmium sulfide, platinum, gold, silica, zirconia, titanium, and others [(3)]. Fungi can produce larger portions of NPs due to they can produce an excessive complete of mycelia and secrete massive totals of proteins which at once translate to greater productiveness of NPs [(5)]. In our study, we evaluated the effects of *Fusarium graminarum* AgNPs in vivo toxicity of the liver and small intestinal of male albino mice.

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Materials and Methods

- ***Fusarium graminarum***

Fusarium graminarum isolated from the decayed banana fruit, it isolate was maintained by serial culturing on potato dextrose agar medium, incubated at 28^{O}C for 4-5 days.

- **Potato dextrose broth medium (PDB)**

Suspend 24 grams in 1000 milliliter distilled water(d.w). Heat to dissolve the medium totally. Sterilize by autoclaving at 15 lbs pressure and temperature 121°C in 15 min. It was cooled down then chloramphenicol antibiotic (250mg /liter) was added. This broth was used for culturing of *Fusarium graminarum* in the extracellular synthesis of AgNPs.

- **Potato dextrose agar medium (PDA)**

It was prepared that 39gm PDA was dissolved in 1000ml d. w. according to the instructions of the manufacturer. Sterilized for 15 minutes by autoclaving at pressure of 15 lbs and temperature of 121 ° C. It was cooled to 45-50oC, and the antibiotic chloramphenicol (250mg / liter) was added to sterile Petri dishes afterwards. *Fusarium graminarum* isolates were cultivated and protected using this medium.

Preparation of *Fusarium graminarum* AgNPs

Fusarium graminarum mycelia used to be inoculated in 250 ml flasks, every flask containing 100 ml of the PDB medium, then incubated for 5 days at 25 ± 2 ° C.Later, mycelia have been accumulated by way of purification via Whatman (No. 42)filter paper and washed thrice with d. w. to dispose of the traces of the medium on fungal biomass. The washed mycelia had been re-suspended in 100 ml d. w, then incubated at 25°C for 24 hours. Once more, mycelia were accumulated by using filtration via. Then, cells filtrate used to be divided into two parts, first one dealt with 1mM of $AgNO_3$ solution and incubated at room temperature, which adjustments color to brown regarded as Positive control, while the second part left except the addition of $AgNO_3$ to the cells filtrate barring a color change viewed as negative control. After adding AgNO3, the finding of AgNPs was mainly done by visual observation of color alteration of the fungal filtrate. A dark brown hue in appearance. The exact size, concentration, crystal morphology, aggregation state, and even bio-conjugation configuration were measured using the particle techniques:

1)Atomic absorption spectroscopy flame 2)UV–Visible spectroscopy

3) X-Ray diffraction 4)Atomic force microscopy

Study design

Forty 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 conditions in the animal houses in the biology department in the college of science/ Mustansiriyah University.A study on acute toxicities of *Fusarium* AgNPs was carried out. It was administered orally (0.1ml/day) in ten mice for three week, AgNPs showed very low toxicity and no death occurred in the mice following the oral administration of the maximum concentration 2.5µg/ml.The mice were divided into two groups. Then the groups were inoculated as a following:

Group 1: twenty mice inoculated orally by stomach tube (0.1ml/day) normal saline considers as a control group.

Group 2: twenty mice inoculated orally by stomach tube AgNPs (0.1ml/day) for three week considers as an AgNPs group.

Histological study

All tissue specimens were obtained after sacrificed mice from 40 mice includes (liver and small intestinal), then they were kept in the fixative solution (formalin10%). After the fixation, sections are processed, embedded in paraffin and 5µm thick glass mounted sections are prepared, which are routinely stained with Haematoxylin and Eosin (H&E)[6].

Results and Discussion

The synthesis of AgNPs by using *Fusarium graminarum* was showed after adding AgNO3 to the filtered cell. The color of the mixture changed from colorless to dark -brown compared with negative control remain colorless.These results corresponding with [7,8] reported the appearance of

brown was a clear indicator of the synthesis of AgNPs in the reaction combination.Determine of *Fusarium* AgNPs surface morphology and sizes were measured, using the AFM. The images of AFM for *Fusarium* AgNPs in figure (1) represented particle size distribution, which is 94 nm in diameter.

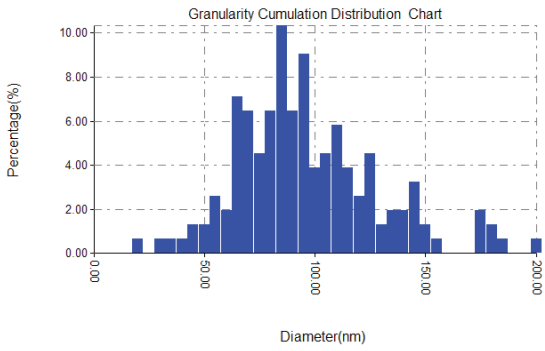


Figure (1): *Fusarium* AgNPs Granularity volume distribution

While in figure (2) showed AFM picture in two dimensions (2D) and three dimensions (3D), it explains structural shape for grains, found that the root mean square is 11.6 nm and average roughness is 9.33 nm.The AFM is a very good technique for measuring surface morphology and fine structure of NPs [9]. The AFM topology is beneficial in revealing the actual dimension and shape of AgNPs [10].

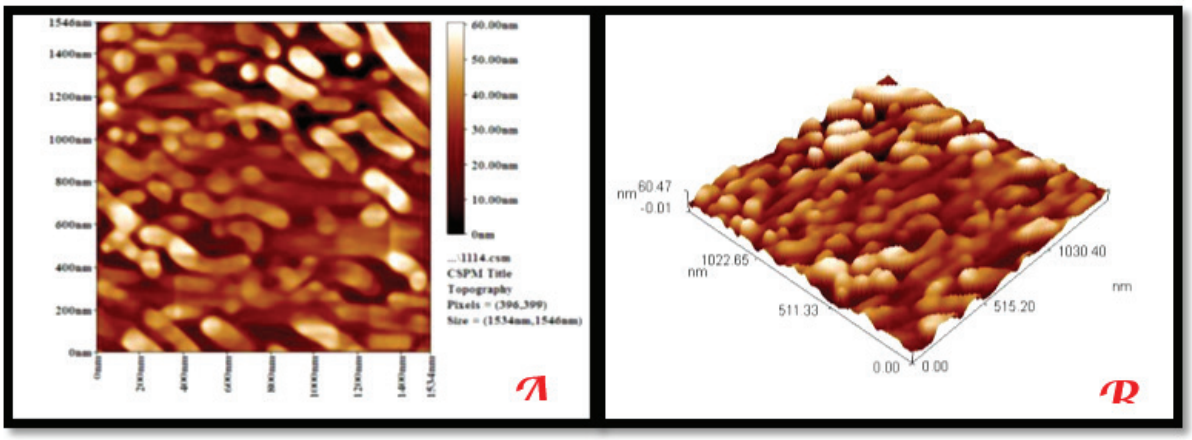


Figure (2): *Fusarium* AgNPs AFM images (A) two dimensions 2D, (B) three dimensions 3D

The XRD pattern of *Fusarium* AgNPs as revealed in fig. (3), the diffraction peaks at 77.31°,64.32°, 44.22°and 38.05° were identical with the (311,220,200,111) the faces of the face-centered crystal cube structure, thus 28.225 nm was average crystallite size.

Our finding corresponds to Shafiq *et al*. [11] who revealed that the XRD diffraction measured in AgNPs resulted in four intense peaks and this further confirms that AgNPs made in extracellular filtration is present in the form of AgNPs. Also, Mahmoud *et al*. [7] reported four distinct diffraction peaks at angles 38.15°, 44.18°, 64.63°, and 77.50° correspond to (111, 200, 220 and 311) planes of the face-centered cubic.

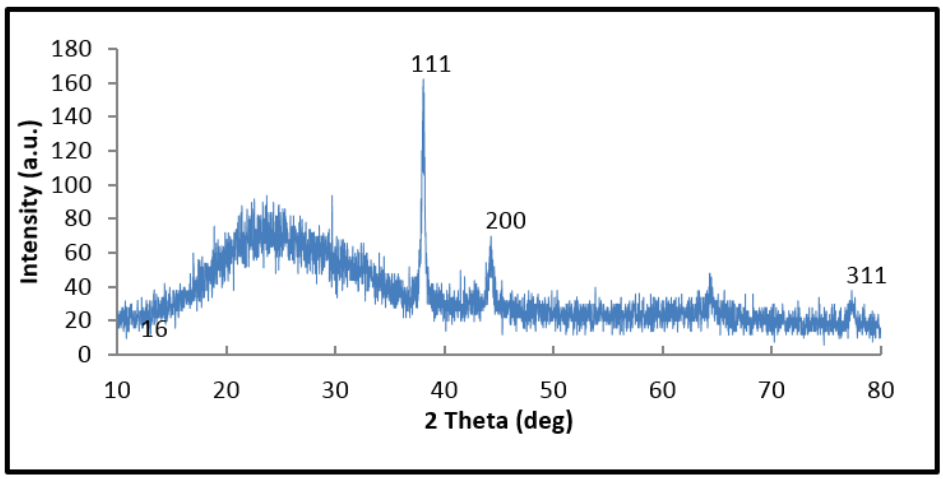


Figure (3): *Fusarium* AgNPs X-Ray pattern

The UV-Visible spectroscopy confirms, existence of *Fusarium* AgNPs by measuring the absorbance of the bio-reduced solution between (300 - 800 nm) wavelengths. Extinction spectrophotometer of UV and visible Vis) light (UV-Vis spectrum) confirmation of the presence of *Fusarium* AgNPs is permitted because of a distinctive Plasmon resonance, figure (4) which revealed a peak absorption at 420 nm.

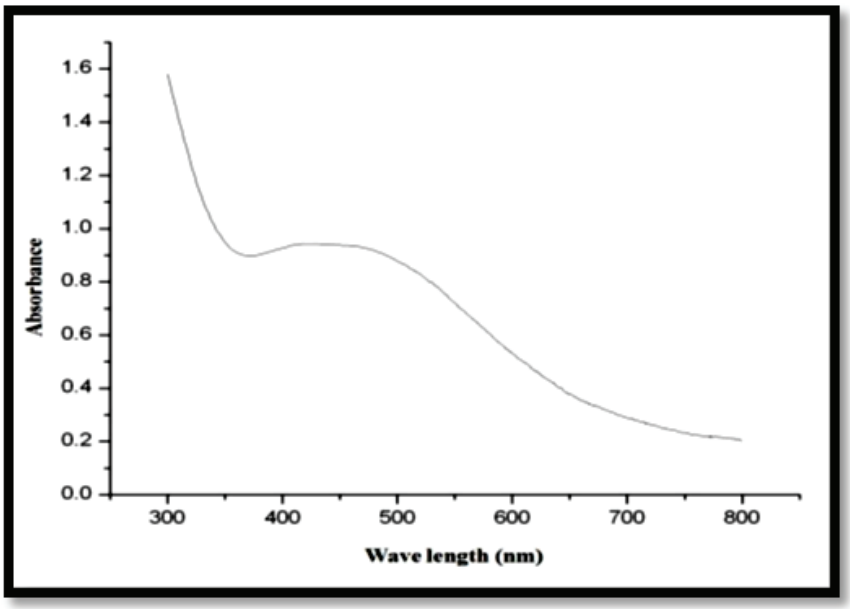


Figure (4): *Fusarium* AgNPs UV-Visible spectroscopy

The results corroborate those of previous studies such as Birla *et al*. [12] who illustrated that the AgNPs is specific when absorption peak at 420 nm. There was one peak refers to the synthesis of spherical NPs, and it is known that there is a very close relationship between the absorption spectra of UV-Vis and the size and shape of AgNPs. Also, Singh *et al*. [13] showed that the production of AgNPs with maximum surface Plasmon resonance peak at 420 nm by using endophytic of *Fusarium spp*.

A. **Histological study**

1. **Liver tissues**

Gross examination of liver obtained from the control group showed normal appearance of hepatocytes, central vein, and sinusoids in all liver mice (Figure 5A).

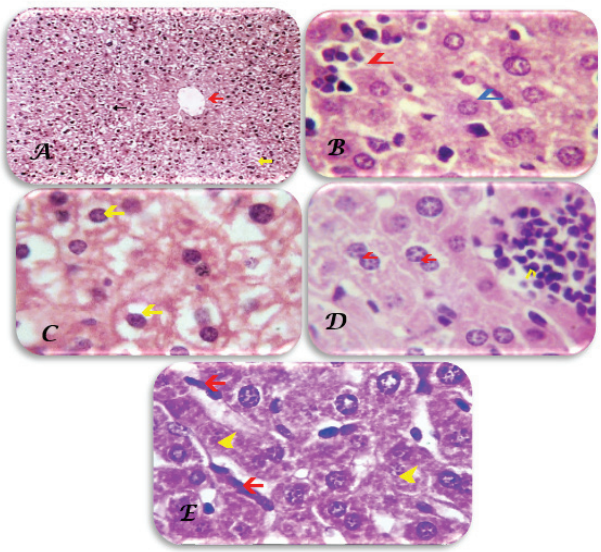


Figure (5): Cross section of the *Fusarium* AgNPs effects after three week in liver of mice detected by H&E staining:

A. control shows normal appearance of hepatocytes (black arrow),central vein (red arrow) and sinusoids (yellow arrow)(X10).**B:** dilation in sinusoids (blue arrow) and infiltration of inflammatory cells(red arrow),**C:** hydropic degeneration in hepatocytes (yellow arrow) ,**D:** proliferation of hepatocytes (red arrows) with aggregation of inflammatory cells (yellow arrow),**E:** proliferation of kupffer cells (red arrows) with degradation of chromatin cells (yellow arrows).B,C,D and E(X40).

Histological changes investigation of liver sections of the treated mice with *Fusarium* AgNPs after three weeks showed, dilation in sinusoids and infiltration of inflammatory cells (Figure 5B) and hydropic degeneration in hepatocytes (Figure 5C). On the other hand, the proliferation of hepatocytes with the aggregation of inflammatory cells (Figure 5D). Also, congestion in sinusoids and proliferation of kupffer cells with the degradation of chromatin cells were observed in many liver sections (Figure 5E).

The liver is the first target organs for *Fusarium* AgNPs, which effect mitochondria by increasing the level of reactive oxygen species as a result production of ATP which may be led to hepatocyte damage [14]. The study of Kawata *et al*. [15] reported when the mice treated with AgNPs revealed lymphocytes infiltration also mitochondrial activation and antioxidant production less effected.

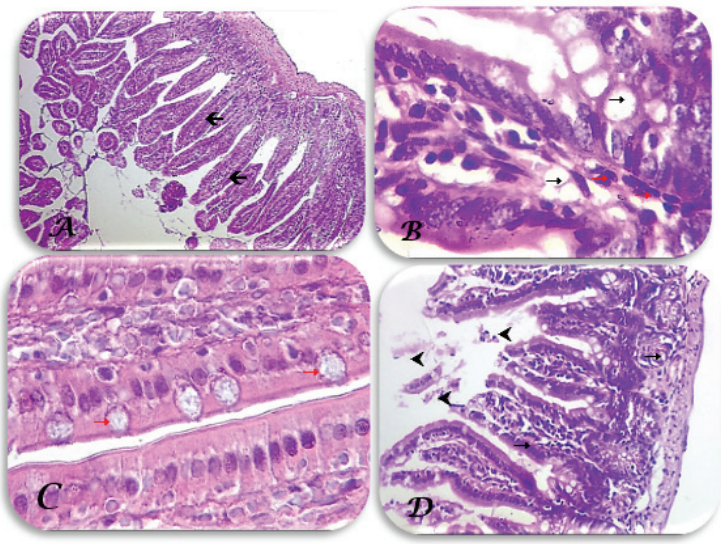
On the other hand, the proliferation of kupffer cells may be to the role of AgNPs ingestion from hepatocytes in tissue [16].This result agrees with Kermanizadeh *et al*.[17] which reveal the role of kupffer cells in the anti-inflammatory response by release different kinds of cytokines. Hydropic degeneration in hepatocytes, it can be explained that the AgNPs affected the mechanism of action of the sodium-potassium pump (Na^{+}-K^{+} pump) that regulates the passage of fluids to and from the cell, which led to the accumulation of fluids within the cell.

2. **Small intestinal**

Histological observation in control mice, normal villi and epithelium cells of the small intestine mucosa (Figure 6A). The following changes were revealed in the sections of small intestine after administration of *Fusarium* AgNPs after three weeks:

a) Hydropic degeneration in epithelium cells of the mucosa and increased number of inflammatory cells (Figure 6B) also, the proliferation of goblet cells in epithelium cells of the intestinal mucosa (Figure 6C).

b) Distortion of mucosal architecture, hemorrhage and sloughing of necrotic villi into the intestinal lumen (Figure 6D).



igure (6): Cross section of the *Fusarium* AgNPs effects after three week in small intestinal of mice detected by H&E staining:

A. control shows normal appearance of the villi and epithelial cells of the intestinal mucosa (black arrows) (X4).**B:**hydropic degeneration in epithelium cells of mucosa (black arrows) and infiltration of inflammatory cells (red arrow)(X40), **C:**proliferation of goblet cells in epithelium cells of the intestinal mucosa (yellow arrow)(X40), **D:** distortion of mucosal architecture, hemorrhage (black arrows) and sloughing of villi into intestinal lumen (arrowheads) (X10).

The results above, show the toxic effect of *Fusarium* AgNPs on the small intestine of mice, as histological changes began with infiltration of inflammatory cells that can be explained by the toxic effect of the AgNPs, which resulted in an inflammatory reaction in the tissues of the small intestine, which led to these cells being attracted to the injury site to remove tissue damaged [18].

On the other hand, the increase in the number and sizes of goblet cells due to exposure to the toxicity of AgNPs that caused damage the epithelial cell microvilli as well as intestinal glands. It may be hypothesized that loss of microvilli reduced absorptive capacity of intestinal epithelium and their irritation to the epithelial lining of the small intestine leading to an increase in their numbers and sizes and it is believed to be a defensive process in order to increase mucus secretion and that reduces the toxic effect on the epithelial lining of the small intestine, finally destruction of the microvilli [19]. Just as the presence, dissociation and separation of epithelial cells lining and necrosis of villi-induced cells the toxicity of the AgNPs, which has an effect on the lining of the blood vessels, results in increased vessel permeability and exit by occurrence, the fluids are out [20]. In addition, in *Fusarium* AgNPs treated mice, microvilli on intestinal absorptive cells were found to have been severely damaged and destroyed. Nanoparticles may be taken across the intestinal barrier, as particles with diameters below 1 μm are particularly susceptible to absorption by the intestinal lymph system [18]. Tang *et al*. [21] also reported that AgNPs cause degenerative changes in some endothelial cells, leading to the loosening of the tight junction between the endothelial cells and AgNPs passing through these crevices. Throughout our study, we observed the structural changes and loss of microvilli, which may give passage to AgNPs for entry into the intestinal wall and consequently into portal circulation and systemic circulation.

Conclusion

We concluded that the size of the AgNPs, due to its ability to enter and translocate within the cell, its toxic effects on the cell and the cell organelle varies. This may therefore be misunderstood that all NPs are toxic and most likely only free NPs, which may penetrate small cell organelles such as mitochondria, may cause adverse effects on health. Therefore,*Fusarium* AgNPs may be less toxic if the exposure time, concentration, or exposure method is less.

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Conflict of Interest:Non

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