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Research Article

BOTANY

Safety assays of biogenic silver nanoparticles synthesized by of *Arthrospira* sp, *in vivo* study

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KEY WORDS

Arthrospira sp.-
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ABSTRACT

Nanotechnology grows rapidly and has potential applications in many areas such as industry, agriculture, business and medicine. So studying its effects and cell toxicity *invitro* and *invivo* was very important recent issue. in this study, silver nanoparticles synthesized by biological ways (green synthesis) using *Arthrospira* sp filtrate showed more safe pattern on the experimental animals according to AgNPs concentration used and this was confirmed using biochemical assays and histology samples of kidney and liver. Biogenic silver nanoparticles recorded safety and environmentally friendship that made it a promising tool in drug delivery in the field of medicine.

1. Introduction

Nanotechnology is a modern technology that studies nanometer sized objects. It is expected that nanotechnology will be developed at different levels such as materials, medical devices and systems. Generally, the synthesized nanoparticles have applications in the field of nanomedicine and this opens the way to develop nanoparticles synthesized by different microbes against various human pathogens and diseases. Nano particles, with dimensions ranging from tens to hundreds of nanometers, have unique

features that differ from bulk material properties. Metallic NPs are usually prepared from noble metals, i.e., Ag, Pt, Au and Pd. Among inorganic agents, silver is the metal of choice in the fields including biological systems as well as living organisms and medicine because it has been used most extensively since ancient times to fight infections and control blight. The Ag- NPs can be exploited in medical and pharmaceutical due to their low toxicity to human cells and high thermal stability (1). AgNPs

play an important role in the field of nanotechnology, including chemical stability, conductivity, catalytic activity, and biological activities such as antibacterial, antifungal, antiviral, and anti-inflammatory activities. Because of their cytotoxic potential, AgNPs have been extensively investigated in cancer research.(2).

The most popular preparation of silver nanocolloids is chemical reduction of silver salts by sodium borohydride or sodium citrate. Most of these reducing agents lead to either environmental toxicity or biological perils; therefore, the trend has shifted to biogenic production of NPs for its advantages (3). The primary requirement of green synthesis of NPs is the metal ion solution and a reducing biological agent. In most of the cases reducing agents or other constituents present in the cells acts as stabilizing and capping agents, so there is no need of adding capping and stabilizing agents from outside. The active ingredient responsible for reduction of Ag⁺ ions varies depending upon organism/extract used (4). The Ag-NPs are preferred option because they are nontoxic to the human body at low concentrations and have broad spectrum antimicrobial properties. The toxicity of nanoparticles depends on many factors as, the inorganic used metal, the concentration of the particles and the capping reducing agent. The results revealed that the antimicrobial activity depends on type of the fabric treatment, size of the synthesized Ag-NPs and the algal species used for polysaccharides extraction (5).

Alumina nanoparticles were added to the cell membrane surface and it was seen that they disturbed cell's power function such as permeability and respiration (6). Also, a study by Kim *et al.* in which mouse lymphoma cells line was used, showed that aluminum oxide NPs (<50nm) cause genotoxic effects in the

form of DNA damage without any mutagenic effects. TiO₂NPs can cause cell damage, genotoxic effects, inflammatory responses and changes in cell signaling (8). TiO₂NPs (5-200 nm) possess toxic effects on immune function, liver, kidney, spleen, myocardium, glucose, and lipid homeostasis in experimental animals, hence they should be used with great care (9). Wilson *et al.* showed that TiO₂NPs caused an increase in reactive oxygen species generation, and a decrease in mitochondrial membrane potential, suggesting mitochondrial damage. In a study of Ahamed *et al.* it was observed that AgNPs had toxicity in a variety of organs, including the lung, liver, brain, vascular system, and reproductive organs. AgNPs may cause induction of reactive oxygen species, oxidative stress, DNA damage and apoptosis (11). On the other hand, Gorth *et al.* exposed silver nanoparticles to *Drosophila* eggs with concentrations ranging from 10 ppm to 100 ppm to investigate the size, chemistry, and agglomeration of the silver particles using transmission electron microscopy, X-ray photoelectron spectroscopy, and dynamic light scattering. The results indicated that, nanoscale silver particles (<100 nm) are less toxic to *Drosophila* eggs than silver particles of conventional (>100 nm) size. In this study, *invivo* experiment was performed to investigate the toxicity of biogenic AgNPs from *Arthrospira* sp. on experimental animals.

Materials and methods

Silver nitrate (AgNO₃) was purchased from Sigma-Aldrich.

Arthrospira sp. were cultured at biopharmaceutical lab. SARTA. Alexandria. Experimental animals were purchased ethically from VACSERA-Giza- Egypt.

Biochemical blood kits

Alanine aminotransferase kit (Biodiagnostic), Albumin kit (Biodiagnostic, Egypt), Alkaline phosphatase kit (Biodiagnostic, Egypt), Aspartate aminotransferase Kit (Biodiagnostic, Egypt), Bilirubin (Total) kit (Diamond, Egypt), Creatinine kit (Biodiagnostic, Egypt) and Total protein. (Biodiagnostic, Egypt).

Methods

1- *Arthrospira* sp culture

Arthrospira sp was cultured on modified Zarrouk medium for 16 days at about $25 \pm 27^\circ\text{C}$ and 2000 LUX under aseptic conditions (13). Cells of *Arthrospira* were centrifuged for filtrate separation for use.

2-Green synthesis of AgNPs using algal free culture

Fifty ml of AgNO_3 (1Mm) were added to one ml of culture filtrate of *Arthrospira* sp (PH= 11), drop wisely, it was shacked for 1 hour (220 rpm) at room temp, then was centrifuged at 10000 rpm for 10 min., the formed pellets were washed 3 times with distilled water before lypholyzation and storing in room temp. till use.

1- Characterization of the synthesized AgNPs (TEM)

Lyophilized samples were studied for the morphology of AgNPs was analyzed using Transmission electron microscopy at an accelerated voltage of 15 kV. The samples surfaces were vacuum coated with gold for TEM.

1-Experimental design (animal grouping):

After an adaptation period of one week, the animals were divided into three groups, each of 5male mice (35-45 gm) were purchased from VACSERA, Cairo, Egypt:

- **Group 1:** normal control group injected with 9% saline as control
- **Group 2:** 5 male group of mice injected intrapretoneial (IP) with (0.3mg/ml) AgNPs nanoparticles dissolved in distilled water as ($7\mu\text{g/g}$) body weight.
- **Group 3:** 5 male group of mice injected intrapretoneial (IP) with (0.15mg/ml) AgNPs dissolved in distilled water as ($3.5\mu\text{g/g}$) body weight.

Animals received the normal dried food. The study lasted for 2 weeks before scarifying under diethyl ether anesthesia, serum samples were collected for different blood biochemical tests.

1- Biochemical analysis

5.1. Determination of alanine amino transferase (ALT) activity

The serum alanine aminotranseferase activity in the sample was determined according to the method of Reitman and Frankel (14) by the kits obtained from Biodiagnostic, Egypt.

5.2.Determination of aspartate aminotransferase (AST) activity

The serum aspartate aminotranseferase activity in the sample was determined according to the method of Reitman and Frankel (14) by the kit purchased from Biodiagnostic, Egypt.

5.3.Determination of alkaline phosphatase (ALP) activity

Alkaline phosphatase was determined by the method reported by Belfield and Goldberg (15) using ALP-kit obtained from Biodiagonestic, Egypt.

5.4. Determination of total protein concentration

Serum total protein concentration was determined according to the method of

Doumas *et al.* (16) using reagent kits purchased from (Biodiagnostic).

5.5.Determination of albumin concentration

Serum albumin concentration was determined according to the method of Doumas *et al.* (16) using reagent kits purchased from biodiagnostic.

5.6.Determination of urea concentration

Urea was determined according to the method reported by Fawcett and Scott (17) using reagent kit obtained from Biodiagnostic, Egypt.

5.6.Determination of creatinine concentration

Serum creatinine concentration was determined according to the method of Schirmeister *et al.* (18) using reagent kits obtained from Biodiagnostic, Egypt.

2-Histopathology

One longitudinal half of each organ (liver and kidney) were fixed at 10% formalin for the histopathological analysis. The tissues were immersed in paraffin where sections of 5 mm were obtained with a standard microtome. The obtained sections were stained with hematoxylin and eosin (H & E), examined microscopically for presence of ant negative features, such as edema, erosion and necrosis compared with control and eventually photographed (19).

Results

1- Green Synthesized silver nanoparticles of *Arthrospira* sp

Silver nanoparticles recovered from filtrate of *Arthrospira* culture after one hour of shaking incubation at 25°C , the

AgNPs particles have different shapes and their size were at the range of (6.5-10 nm) as TEM showed in photo 1.

2-Invivo toxicity of AgNPs of *Arthrospira* sp.

2.1. Effect of AgNPs on on serum aspartate amino transferase (AST), alanine amino transferase (ALT) and alkaline phosphatase (ALP) activities (Table1 and Figure 1)

The results represented in table 1 and figure1 revealed that first group treated with dose (3mg/ml) of AgNPs showed significant elevation in ALT, AST and ALP from control levels with 32.9%, 21.5% and 12.8% respectively . However, second group treated with (1.5mg/ml) AgNPs produced insignificant difference from control levels.

2.2. Effect of AgNPs on on serum bilirubin (Table 2 and Figure 2)

The results represented in table 2 and figure 2 showed that first group treated with dose (3mg/ml) of AgNPs showed significant elevation of bilirubin with 47.3% from control level. However, second group treated with (1.5mg/ml) AgNPs showed less significant difference from control levels by 21%.

2.3. Effect of AgNPs on protein and albumin profile of serum of mice (table 3 and figure 3)

The results represented in table 3 and figure 3 revealed that first group treated with dose (3mg/ml) of AgNPs showed significant elevation in total protein (9.6) mg/dl from control level (6.5) mg/dl with 47.6% percentage. However, second group treated with (1.5mg/ml) AgNPs showed (7.6) mg/dl insignificant difference from control level value, while albumin level was reduced in first group (5.3) mg/dl with significance reduction about 37.6 % with no significant reduction in second group compared with the control group.

2.4. Effect of AgNPs doses on serum urea and creatinine activities (Table 4 and Figure 4)

The results represented in table 4 and figure 4 revealed that first group treated with dose (3mg/ml) of AgNPs showed significant reduction in creatinine level from control level with 26.3 percentage. However, second group treated with (1.5mg/ml) AgNPs produced insignificant difference from control levels, while urea level was elevated with significance in first group and second group compared with control group with 27.7 % and 9.1% respectively.

2- histology of kidney and liver of tested animal groups

Kidneys and livers of the tested animal groups were isolated and preserved in formalin, sections of kidneys and livers were stained with H&E staining after pooling to examine the differences produced after AgNPs injection for two weeks. As shown in photos (2,3,4 and 5) the high dose of AgNPs caused little inflammation in both kidney and liver of the tested animals in group 2 compared with animals of group 1 .

Discussion

Metal nanoparticles used recently almost in all fields, this is the era of nanotechnology. Nanotechnology played an important role in medicine in drug delivery for its specific characters, so many researches recently focused on it and its effects and toxicity *invitro* and also *invivo*. Toxicity of metal nanoparticles and AgNPs depends on the nanoparticles size and concentration (20) as also shown by this study. Additionally, it was observed that the deposition of nanoparticles inside the nucleus of the cells can cause DNA damage and chromosomal aberrations. Nanoparticle deposition in the central nervous system has negative effects on controlling the cardiac rhythm, respiration and body

movements. Moreover, the exposure of AgNPs caused hyperemia in different parts of the body thus edema and necrosis occur (20) In a study of Ahamed *et al.* negative effects of copper oxide nanoparticles such as cytotoxicity, oxidative stress, DNA damage were investigated. The results indicated that exposure to CuNPs caused DNA damage in human lung epithelial cells by lipid peroxidation and oxidative stress (21).as confirmed with our study, green synthesized silver nanoparticles safe pattern depends on nanoparticles size, concentration and metal type.

Conclusion

Biogenic silver nanoparticles were safe on experimental animals and its safety depends on the concentration used. It is promising alternative for chemically synthesized nanoparticles in many fields such as medicine.

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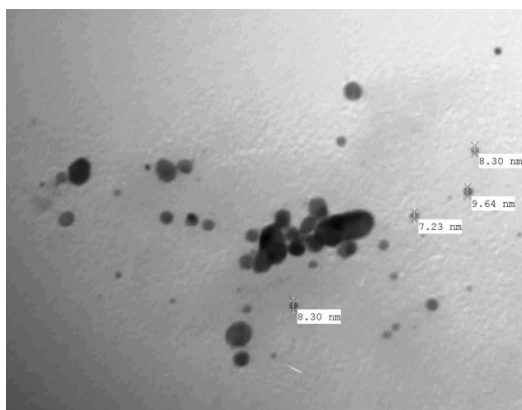


photo 1: TEM of AgNPs of *Arthrospira* sp cell free culture showing the size and shape of produced particles

Table 1: ALT, AST and ALP levels in control, group 1 and group2

Test/G	Control	Group 1	Group 2
ALT U/I	28.2 ^a	37.5 ^b	31.3 ^a
AST U/I	51 ^a	62 ^b	55.2 ^a
ALP U/I	344 ^a	388.2 ^b	373.4 ^a

- Values with the same capital letter in the same row showed insignificant difference (at $P \leq 0.01$).
- Values with the same small letter in the same column showed insignificant difference (at $P \leq 0.01$).

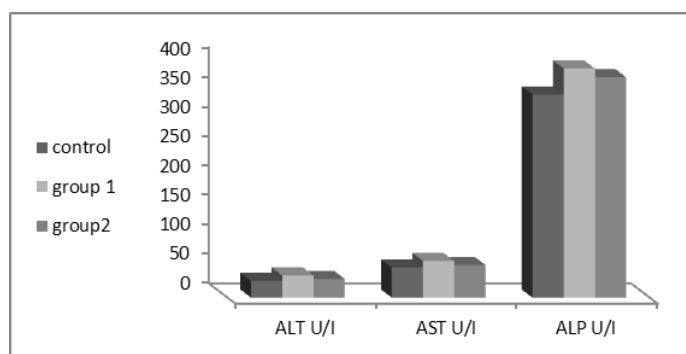


Figure 2: ALT, AST AND ALP levels in U/I for control, group 1 and group 2.

Table 2: bilirubin levels in control, group 1 and group2.

Group	Bilirubin mg/dl
control	1.9 ^a
group 1	2.8 ^b
group 2	2.3 ^c

Values with the same capital letter in the same row showed insignificant difference (at $P \leq 0.01$).

Values with the same small letter in the same column showed insignificant difference (at $P \leq 0.01$).

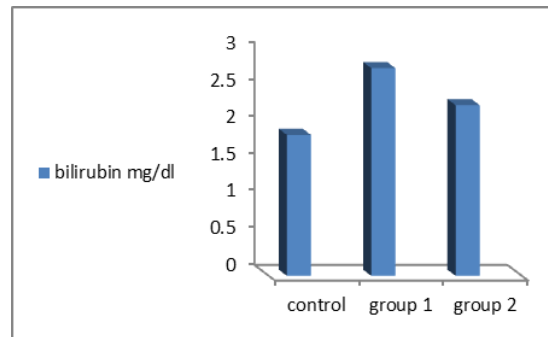


Figure 2: Bilirubin levels in mg/dl for control, group 1 and group 2.

Table 3: protein profile of control, group 1 and group2.

Group	protein g/dl	Albumin g/l
Control	6.5 ^a	8.5 ^a
group 1	9.6 ^b	5.3 ^b
group 2	7.6 ^a	7.5 ^a

Values with the same capital letter in the same row showed insignificant difference (at $P \leq 0.01$).

Values with the same small letter in the same column showed insignificant difference (at $P \leq 0.01$).

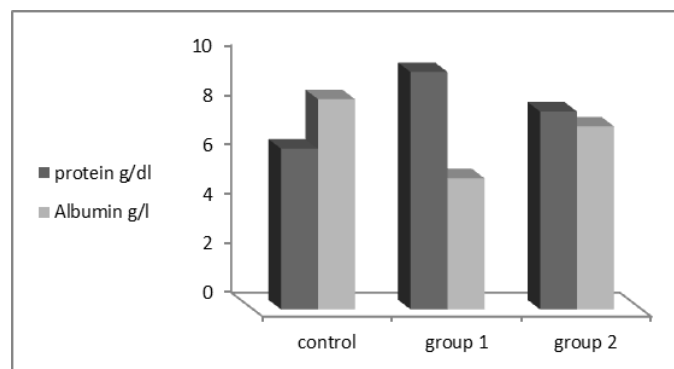


Figure 3: protein profile for control, group 1 and group 2 as total protein and albumin.

Table 4: creatinine and urea levels of control, group 1 and group2.

Group	creatinine g//dl	Urea g/dl
Control		
group 1		
group 2		

Values with the same capital letter in the same row showed insignificant difference (at $P \leq 0.01$).

Values with the same small letter in the same column showed insignificant difference (at $P \leq 0.01$).

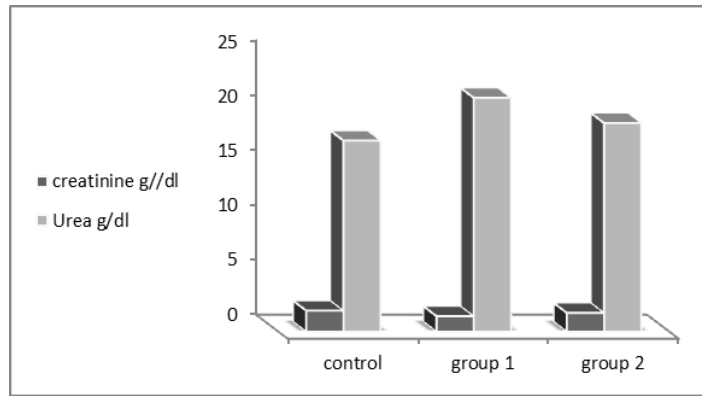


Figure 4: creatinine and urea levels for control, group 1 and group 2.

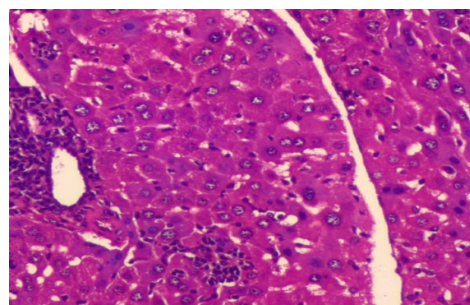
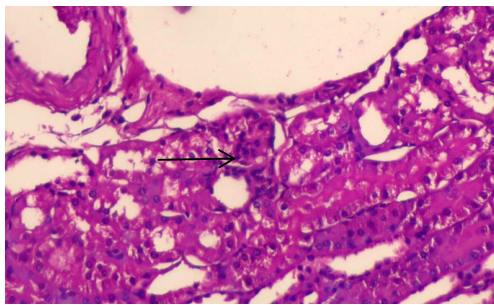


Photo 2 and 3: H&E staining of kidney and liver in mice treated with AgNPs of *Arthrospira* sp.(group 1) Indicating start of degeneration of kidney tubules and liver cells inflammation.

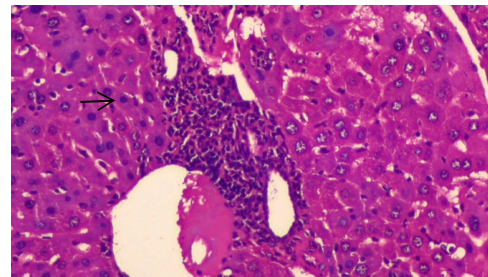
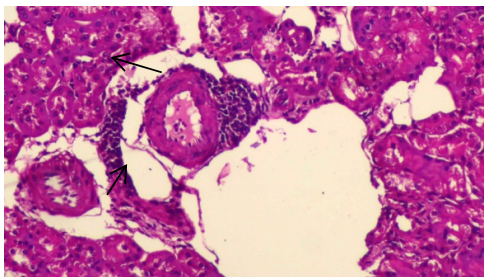


Photo 4 and 5: H&E staining of kidney and liver in mice treated with AgNPs of *Arthrospira* sp.(group 2) Indicating perivascular focal inflammation