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Cytotoxicity assessment of silver nanoparticles on liver and spleen of females' rats

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Abstract

Silver nanoparticles (AgNPs) is the most important nano molecules that have many medical, industrial, and cosmetic application. We study the effect of these particles on the most vital organs such as the liver and spleen in Wister rats. AgNPs are characterized by using a scanning probe microscope device (SPM). Twenty females were divided into two groups, the control, and the treated group. All treated female's gavage 20mg/ml of AgNPs suspension orally for 29 days. The weight of females was 130±5 g. The liver and spleen were used in this study after staining by H&E. The result showed few intracytoplasmic fat vacuoles on liver tissue, on the other hand, the result displayed brown pigment in the sinusoids. The result showed found marked vascular congestion in liver tissue after being treated with AgNPs compared to the control group. The histological examination of the spleen in treated rats showed the presence of brown pigment in the red pulp of the spleen and congestion, and hemorrhage in treated rats. The result showed brown pigments deposited mainly in red pulp compared to the control group. The result presented that this nanoparticle could penetrate the membranes and reaches into many body organs because can penetrate the brain barrier, bloodstream, and many barriers to the harmful effect of acute exposure or chronic exposure to these nanoparticles.

Keywords: Females rats, liver, spleen, silver nanoparticles, cytotoxicity.

Introduction

Recently, nanotechnology has displayed interesting properties in many fields, especially in the medical field, through new constructions with many beneficial possibilities (1,2). Due to the aspect ratio of nanomaterials, they have been developed as antimicrobials, antifungals, and increased antibiotic activity. The metal nanoparticles (NPs) in the nanoscale organization show significant modifications in the electrical, optical, and catalytic characteristics (3). Silver nanomaterial (Ag) is naturally one of the most vital available antiseptic materials. AgNPs are primarily bactericidal effects because of their powerful interaction with thiol groups that are in the respiratory enzymes of the bacterial cell and have structural protein that interactions preferably bind with the nucleic acid bases of DNA to avoid the replication process. The properties of nanosilver particles depend on their shape, size, surface modification, aggregation, and morphology (4, 2).

On the other hand, the risks postured by stabilizer coated AgNPs remain to be unclear and assessing their toxicity for an understanding of their safety involved for use in various applications (5).

The major mechanism of action of these nanoparticles is expected to be a generation of nonspecific reactive oxygen species (ROS). The

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structural injury or damage of the cell's mitochondria can also release more than ROS, this oxidative stress leads to undergo apoptosis in the cell (6,7). Because of the extensive use of AgNPs in many products so, they are either directly entered the body by ingestion or through environmental contamination. These particles can enter through numerous routes to exposure, such as through the gastrointestinal or the respiratory system, or even by injury and reach the bloodstream or major organs. Moreover, these AgNPs can pass the blood-brain barrier (BBB) by transcytosis of capillary endothelial cells to enter several tissues such as the spleen and liver and other organs to induce further damage (8).

Material and methods

AgNPs preparation

AgNPs were used in this study, purchased as a grey-black solid powder (purity 99.9%, apparent density: 0.97g /ml, tap density: 2.16 g/ml, and CAS NO.: 7440-22- 4) with an average diameter of (50.28) nm. The AgNPs stock solution was prepared by suspending the calculated weight of AgNPs powder in a certain volume of deionized distilled water D.D.W in a sterile glass universal tube. The suspension was exposed to the ultrasonication technique by ultrasonic water bath for 2-3h in the dark and under biological safety conditions.

Properties of AgNPs

Silver nanoparticles are characterized by using a scanning probe microscope device (SPM). The granularity cumulation distribution report showed the average of AgNPs diameter was 50.28 nm and the examined sample showed these particles' grains as aspherical in shape (Figure 1).

Avg. Diameter:50.28 nm

<=50% Diameter:50.00 nm

<=10% Diameter:20.00 nm

<=90% Diameter:70.00 nm

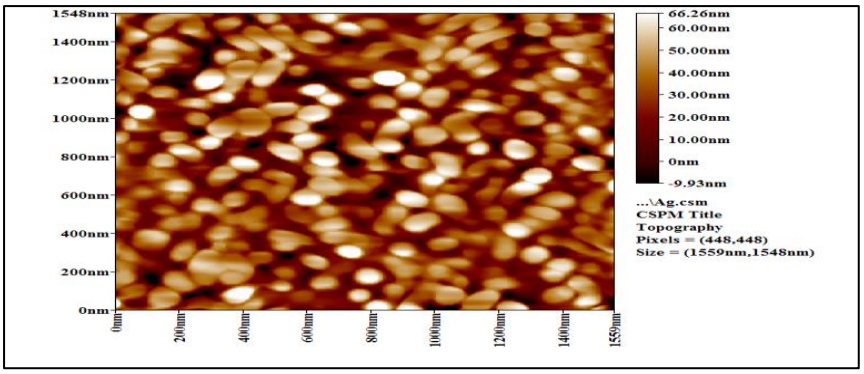

Figure 1: spherical nanoparticles shape by using scanning probe microscope device

Animal housing

All twenty animals in the experiment were purchased from the biotechnology research center/ al-Nahrain University. The animals were performed at the animal house in the biology department in clean steel cages. In the present study, female Wistar rats of 5–8 weeks and about 130–135 g in weight. Animals were maintained on standard pellet feed and water ad libitum. The rats were weighed before administration of AgNPs suspension and divided into control and treatment groups, such that the average weight of the body in each group was similar.

Administration of AgNPs

AgNPs were gavage (2 ml) daily for 29 days as an aqueous suspension orally at concentration 20

mg/Kg/ B.wt. The experimental design for this study included dividing the females into two groups, the treatment and control group (the treated group included 15 females and the control group 5 animals). All the animals were weighed after administration. At the end of administration days, each animal was sacrificed by diethyl ether (9).

Histology examination

The tissues and organs were removed surgically, then the organs were washed in isotonic normal saline. Major organs such as the liver and spleen were fixed in formalin (10%) neutral buffer. The organs embedded in the paraffin casket were then stained by using eosin and hematoxylin (E&H) stain.

Further study was examined microscopically for observed histological changes (10).

The results

The control groups

1-liver

The liver is considered one of the most important body organs, which has many important and vital functions as the metabolism of drugs and alcohol, and the purification of the body from toxins (11).

The normal histological section in the liver shows a normal hepatic lobule. The central vein is surrounded by normal hepatic cells separated by normal blood sinusoids (Figure 2) (H&E 20X).

2- spleen

The spleen plays many supporting roles in the body. It acts as a filter for blood as a part of the immune system. As the largest lymphoid organ in the body, the spleen exerts both local and systemic effects on immune cell response. Old red blood cells are recycled in the spleen, and platelets and white blood cells are stored there. The spleen also helps fight certain types of bacteria that cause pneumonia and meningitis (12).

The normal histological section of the spleen includes two mean compartments of the spleen, the white bulb (including the marginal zone) and the red bulb, which are vastly different in their architectural texture (Figure 3).

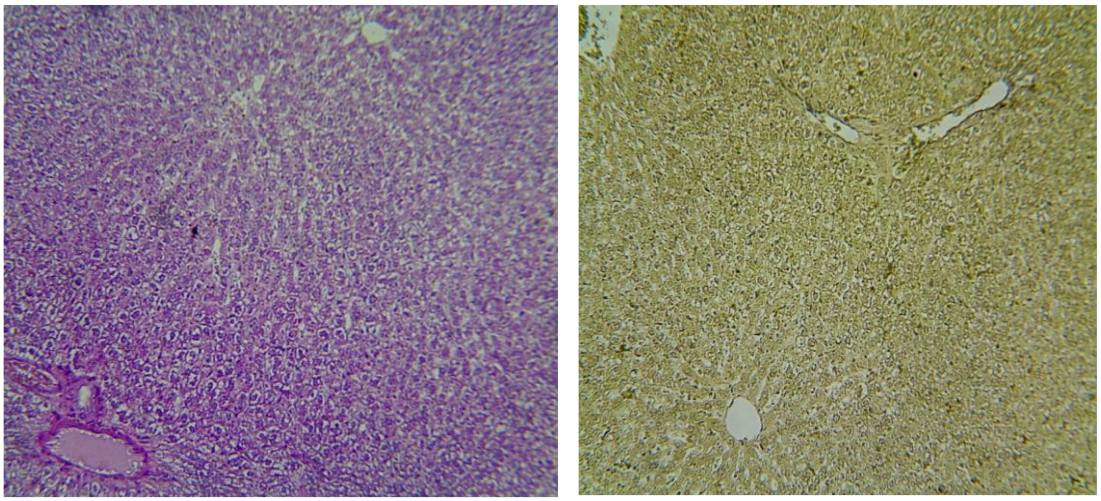

Figure 2: liver tissue from the control group shows normal hepatic lobule. The central vein is surrounded by hepatic cells separated by blood sinusoids (H&E 20X).

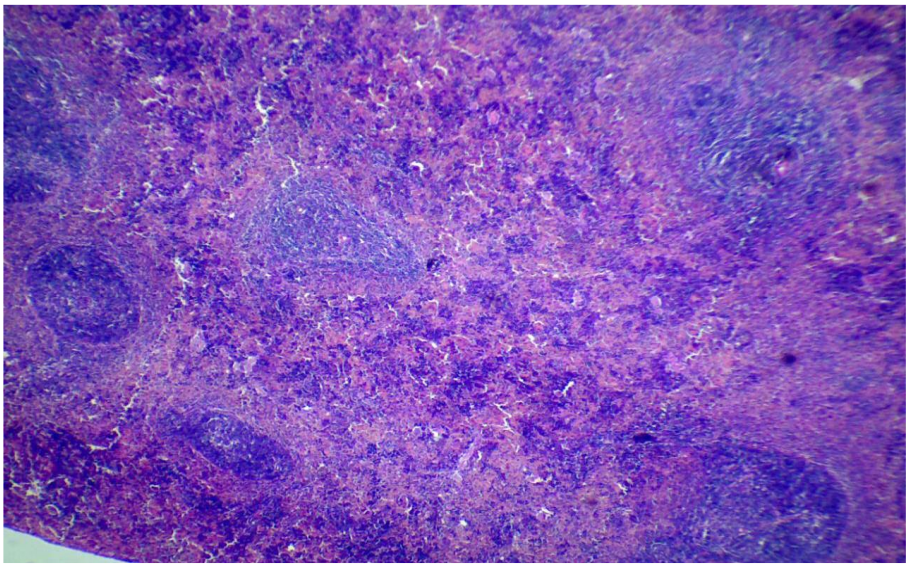

Figure 3: Normal histological section of the spleen shows two mean compartments of the spleen, the white pulp (including the marginal zone) and the red pulp, which are vastly different in their architecture texture (H&E 20 X).

Treated groups

1—liver

Silver nanoparticles AgNPs are shown to penetrate and translocate to the bloodstream by inhalation and ingestion, and such studies demonstrate that the liver is one of the most important organs for accumulation. After orally exposure AgNPs at concentration 20 mg/kg/B. WT the result showed few intracytoplasmic fat vacuoles (red arrow) on liver tissue (Figure 4). On the other hand, the result showed brown pigment in the sinusoids (figure 5).

Further, the result appears found marked vascular congestion in liver tissue after being treated with AgNPs as shown in figure 6. This nanoparticle can be produced a harmful effect on the hepatocyte the basic unit of the liver. The section showed swollen hepatocytes (hydropic degeneration), necrotic hepatocytes, hypertrophied hepatic nuclei, hypertrophied endothelial and Kupffer cells, and dilated sinusoids (figure 7).

The histological sections of hepatic cells from the treated group also showed containing numerous fat globules with inflammatory cell infiltration (lymphocyte & granulocytes) (figure 8).

2-Spleen

The histological section of the spleen in treated rats with AgNPs after 29 days of administration showed the presence of brown pigment in the red pulp of the spleen compared to the control group (Figure 9).

Other effects of these nanoparticles on spleen tissue include congestion and hemorrhage in treated rats (figure 10) and showed brown pigments deposited mainly in red pulp compared to the control group (figure 11).

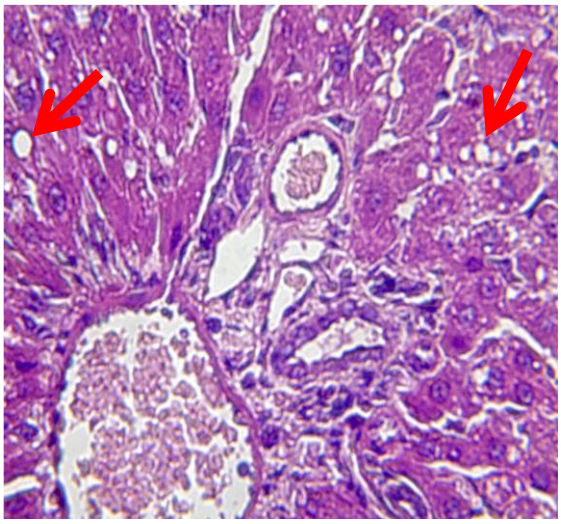

Figure 4: Liver section of treated rat shows few intra cytoplasmic fat vacuoles (red arrow) after exposure of 20 mg/kg AgNPs

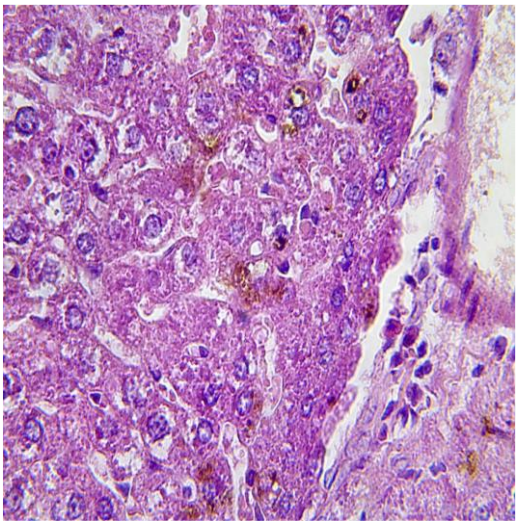

Figure 5: Liver section of treated rat shows brown pigment in the sinusoids after exposure of 20 mg/kg of AgNPs

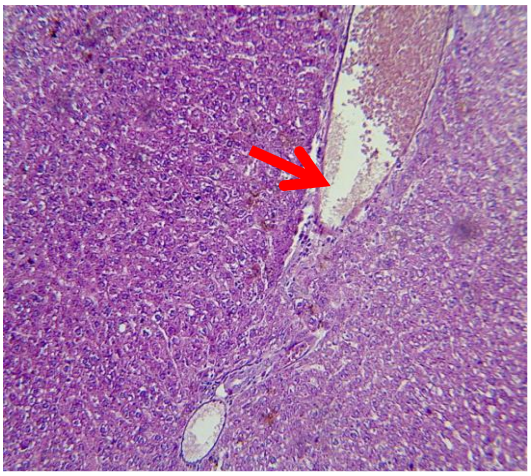

Figure 6: Liver tissue from treated group showing marked vascular congestion (Red arrow) (H&E 20X)

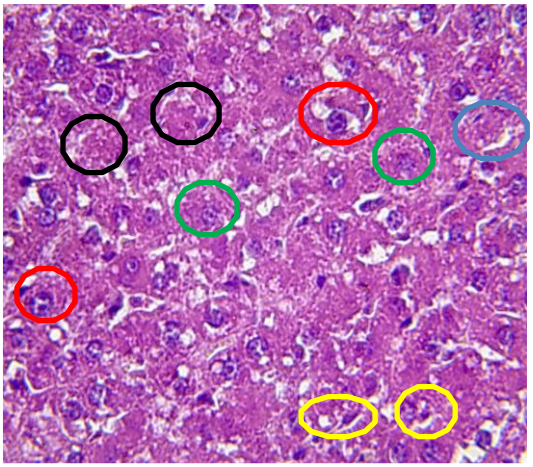

Figure7: liver tissue from the treated group showing swollen hepatocyte (hydropic degeneration) (green circle), necrotic hepatocyte (black circle), hypertrophied hepatic nuclei (red circle) hypertrophied endothelial & Kupffer cells (yellow circle & dilated sinusoids (blue circle). (H&E 40X)

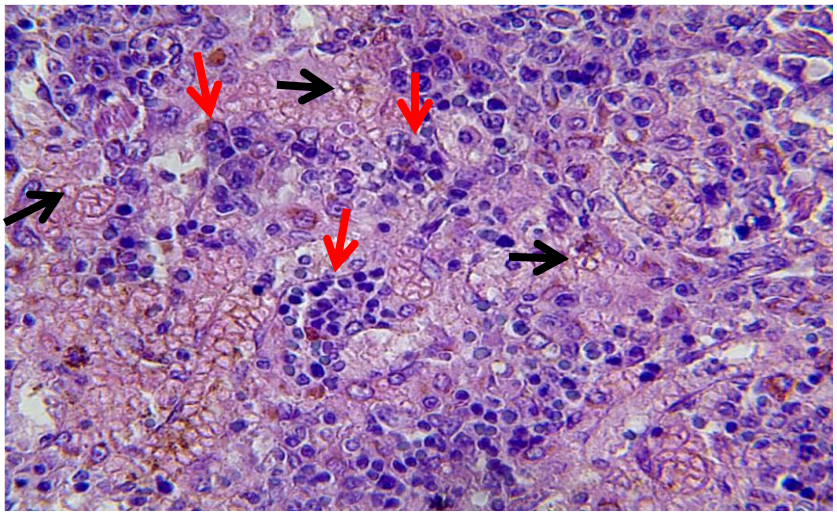

Figure 8: Histological sections of hepatic cell from the treated group containing numerous fat globules (black arrow) with inflammatory cells infiltration (lymphocyte & granulocytes) (red arrow) (H&E 40X)

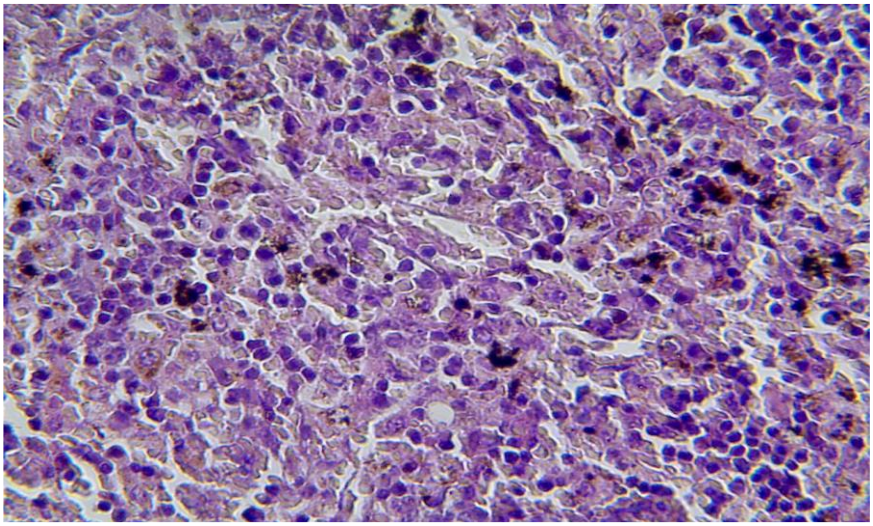

Figure 9: Spleen section in treated rat shows the presence of brown pigment in the red pulp

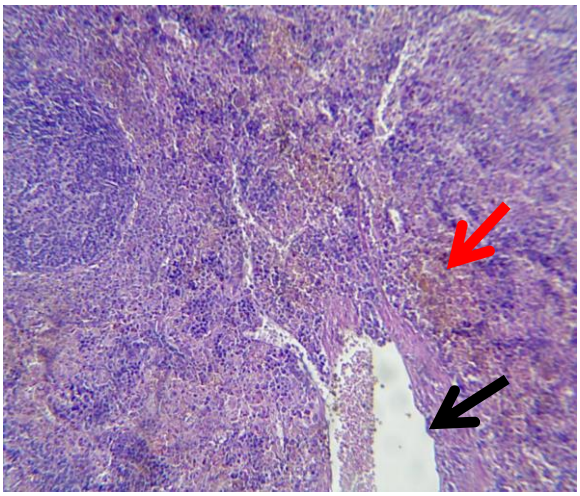

Figure 10: Spleen from treated rat with congestion (black arrow) & hemorrhage (red arrow)

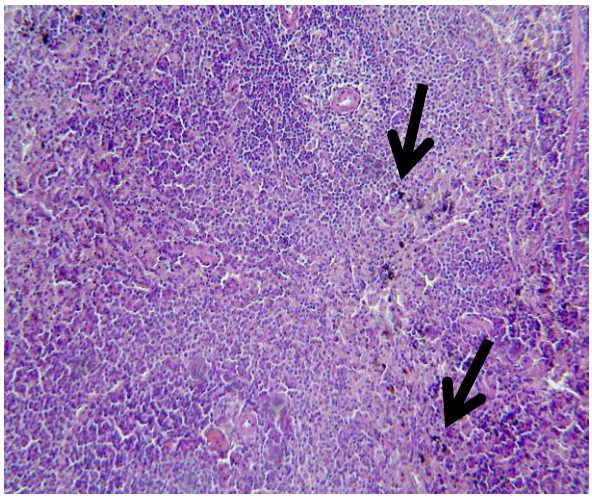

Figure 11: Spleen from treated rat with brown pigments deposit mainly in red pulp (black arrows)

Discussion:

Silver nanoparticles (AgNPs) are presently one of the most frequently used nanomaterials in consumer, industrial and biomedicine applications. However, they remain one of the most controversial research areas regarding their distribution, dissolution, and their toxicity to biologicals. Therefore, the present work investigated the potential toxicity of silver nanoparticles on the structure of rat spleen and liver using different doses, with further assessment of the underlying mechanisms.

Previous studies have shown that when the liver is exposed to a concentration of 0.5 mg/kg of silver nanoparticles, it allows signs of necrosis in certain areas of the liver. As for the portal trades of the liver lobes, the cells adjacent to it showed abnormalities, and these abnormalities are at the level of nuclear material. In addition, studies confirm irregular staining of the cytoplasm of hepatocytes, as well as the appearance of signs of sinusoidal degeneration. The explanation for this is due to the dispersal of the chromatin material and its pushing near the plasma membrane, and this is consistent with the results of our research and referred to it (14).

There are other studies on the effect of silver nanoparticles on liver tissue when exposed to certain doses, which led to pathological changes in the liver in the form of multiple foci of necrosis, fibrosis, and inflammatory cell infiltration compared to the control group This is what he indicated (15).

Studies have shown that there are histopathological changes in the spleen architecture and its structure, due to the presence of silver nanoparticles in spleen cells, as a result of changes in splenic lymphocytes and a reduction in the size of splenic vesicles, in addition to the presence of programmed death of some cells and the presence of cell vesicles (16).

In line with the results that showed us that the damage to the spleen tissue is due to the amount of dose given by silver nanoparticles, explained this phenomenon of toxicity depends on the time and sedimentation of the substance in samples of the spleen and this is consistent with our results and their interpretation (17)

Another study observed the steric degeneration of the spleen parenchyma in samples that were exposed to silver nanoparticles. These microparticles stimulate the formation of autophagosomes, which contain cellular debris and degraded materials as well as lipid droplets, which leads to a change in the intracellular balance.

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