

The Effects of Different Concentrations of Silver Nanoparticles on the Kidneys of Male Albino Mice

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Abstract

Silver nanoparticles (AgNPs) are one of the most abundantly used nanomaterials. Following exposure, AgNPs can accumulate in various organs including kidneys. This study aimed to determine the effects of increased doses of AgNPs on the kidneys of adult male albino mice. Sixty adult white male mice (20-25 g) were randomized divided equally into four groups (15 mice each): control group (saline solution), low-dose group (LD AgNPs) was intraperitoneal (i.p.) injected with 1 g/ kg, medium dose group (MD AgNPs) was i.p. injected with a dose of 2 g/ kg and high dose group (HD AgNPs) was i.p. injected with a dose of 4 g/ kg. Samples were taken from each group after one week, two weeks and four weeks of injection. Then blood was collected from all animals for serum analysis of kidney functions (serum creatinine, uric acid and blood urea nitrogen) at the end of each period and the kidneys were examined microscopically. The results showed impaired kidney function as revealed by increased in sera levels of creatinine, uric acid and blood urea nitrogen in rats treated with AgNPs. The study of light microscopy showed tissue changes in the kidney membranes including programmed death and focal necrosis. One of the most significant effects was changes in nuclei that appeared either enlarged or bilateral in the kidney cells. The biochemical and structure were more prominent by increased dose and prolonged duration. The study concluded that despite the benefits of silver nanoparticles, it is important to test the safety of the use of different doses to determine the safe dose, especially on vital organs such as kidneys.

Keywords: Silver nanoparticles; mice; kidneys; nephrotoxicity; structure.

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1. Introduction

Silver nanoparticles (AgNPs), also known as nano-silver, have been used into numerous food substances and medical applications [1]. AgNPs had strong antimicrobial [2], antifungal [3] and antiviral [4] characteristics. However, in spite of their usefulness, increasing evidence suggests the side effects of AgNPs in animals and human. Silver nanoparticles are nanoparticles of silver with size range from 1-100 nm. Various shapes of nanoparticles can be made depending on application at hand. Commonly used shapes are spherical nanoparticles but octagonal, diamond, and thin sheets are also used [5].

Researchers showed that the side effects of these nanoparticles are depend on the dose and size. It has been reported that AgNPs are toxic than other metal nanoparticles, as aluminum, nickel, iron, and manganese [6]. The side effects of free silver ions on humans and animals are permanent skin bluish-gray discoloration (argyria) or eyes (argyrosis), liver and kidney damage; eye, skin, respiratory tract, and intestinal tract irritations; and untoward changes in blood cells when given through oral, inhalation or subcutaneous routes [7, 8].

Different types of AgNPs-induced toxicities have been reported in *in vitro* and *in vivo* researches. Earlier *in vitro* studies have reported increased cytotoxicity [9], genotoxicity [9, 10], oxidative stress and mitochondrial damage [11] following AgNPs exposure. For the evaluation of nanomaterials, *in vitro* assays may not reflecting *in vivo* nanomaterials toxicity due to the complex nature of nanomaterials and the complicated processes of uptake, deposition and distribution of nanoparticles in the whole body [12]. *In vivo* studies demonstrated that AgNPs enter the blood circulation and accumulate in organs including the liver, brain, kidneys, lungs, spleen and testes [6, 8, 13]. Despite their widespread use, there is only limited data on safety, toxicology and exposure of silver nanoparticles.

This experimental study aim to evaluate the potential toxic effect of intraperitoneal injection of different doses of silver nanoparticles on the renal ultrastructure and function of male albino mice.

2. Materials and Methods

2.1 Chemicals

Ascorbic acid, silver sulfate, sodium borohydride, tri-sodium phosphate 1-hydrate were obtained from Sigma Aldrich, USA. Epoxy resin kit, glutaraldehyde and osmium tetra oxide were purchase from SPI, USA. Creatinine, urea nitrogen and uric acid kits were purchase from Siemens Com., Munich, Germany.

2.2 Silver nanoparticles (AgNPs)

AgNPs were obtained from Sigma Aldrich, USA (cat. 576832), with particle size of less than 100 nm, 99.5% purity and 0.2% PVP as dispersant. Size distribution in the medium (double distilled water), polydispersity, agglomeration and charge on these particles were assessed using dynamic light scattering (DLS). AgNPs were suspended in double distilled water (DDW) at various concentrations ranging from 5µg/mL to 40 mg /mL, sonicated (Vibra- CellTM, Sonics & Materials, Inc. USA) for 10 minute on ice at 4°C and analyzed by DLS

(Zetasizer Nano-ZS, Malvern Instruments, Malvern, UK). The AgNPs were distinguished by using high resolution-transmission electron microscopy (HR-TEM). Silver Nano powders were dissolved in 70% Ethyl alcohol solution using a sonicator (BRANSON 1510). The suspended Ag NPs were then mounted on carbon coated copper grid. The shape of coated samples was seen by high resolution-transmission electron microscopy (HR-TEM; JEM-2100F, Jeol, Tokyo, Japan) instrument operating at an accelerating voltage of 200 KV [14]. The particle size shape and surface morphology was confirmed using Transmission electron microscope (TEM).

2.3 Animals

Sixty male albino mice of the SWR strain, weighing 22.0 – 25.0 g were used in the present research. The experimental animals were purchased from the Experimental Animal Unit of King Fahd Medical Research Center, King Abdul-Aziz University, Jeddah, Saudi Arabia. The mice were distributed into four groups (fifteen mice per group) and were housed in standard plastic cages at an environmentally controlled (temperature 25°C, relative humidity 55%, 12- h light /12- h dark cycles). Standard diet, commercial feed pellets, and tap water were freely available. The procedures used in this study are approved by Animal Ethics Committee of King Abdul Aziz University.

2.4 Experimental protocol

After one week of acclimation, the mice were randomly sorted into 4 equal groups as followings: control (saline solution), low-AgNPs (1g/kg), middle- AgNPs (2 g/kg), and high- AgNPs (4 g/kg.). The animals were kept fasting for 12 hours before injected. The mice were injected intraperitoneal with one dose of AgNPs. After administration; third of the animals were anesthetized and sacrificed on day 7, third on day 14 and third on day 28 after injection. Before scarification venous blood samples were collected from retro-orbital veins. The kidney specimens were rapidly collected from each group and there were kept in 10% formalin for light and 2.5% glutaraldehyde for electron microscopic examination [15].

2.5 sampling and analysis

Mice were anaesthetized by using diethyl ether and venous blood samples were collected from retro-orbital veins in non-heparinized tube after 1st, 2nd and 4th weeks. The blood was centrifuged at 3000 rpm for 10 min and sera were collected and kept at -80 °C. The creatinine method employs a modification of kinetic Jaffe reaction. This method reported to be less susceptible than conventional method to interference from non-creatinine, Jaffe-positive compound [16]. The uric acid method is modification of uricase method that was reported by Bulgar and Johns [17], later modified by Kalckar [18]. Measurement of uric acid by estimated the absorbance loss at 293 nm following uricase treatment is more specific than other indirect methods. Urea is the major nitrogen-containing metabolic product of protein breakdown in humans. The principal utility of urea nitrogen determination lies in conjunction with measurement of creatinine in serum of plasma to discriminate between prerenal and postrenal azotemia. Urease specifically hydrolyzes urea to form ammonia and carbon dioxide. The ammonia is used by glutamate dehydrogenases enzyme (GLDH) to reductively aminate α -ketoglutarate (α -KG), with reduced nicotinamide-adenine dinucleotide (NADH) oxidation. The change in absorbance at 340 nm due to

NADH disappearance is directly proportional to urea nitrogen concentration and is measured using a biochromatic (340, 383 nm) rate method.

2.6 Histopathological examination

At the end of experiments, animals were killed and the kidneys were excised and washed then fixed in 10% buffered formal saline (10% formalin with 0.9% NaCl). Samples were dehydrated and embedded in paraffin wax. Sections 4-5 μm thick were stained with haematoxylin and eosin (H&E) accordance to Drury and Wallington [19] and examined under Olympus trinocular microscope (BX-51). Sample photomicrographs were taken primarily under different power of magnification.

2.7 Statistical analysis

Statistical analyses were made using SPSS version 20 (SPSS, Chicago, IL). All data was expressed as mean and standard deviation (SD). For significance verification by groups in the same week versus control, one-way ANOVA was performed, followed by LSD test. For significance verification by weeks in the same group versus 1st week, paired student "t" test was performed. *P* value of <0.05 was considered statistically significant.

3. Results

3.1 Silver Nanoparticles

Transmission electron microscopy (TEM) is specific method for imaging nanomaterials to obtain quantitative assessment of particles and/or size and shape as shown in Figure (1).

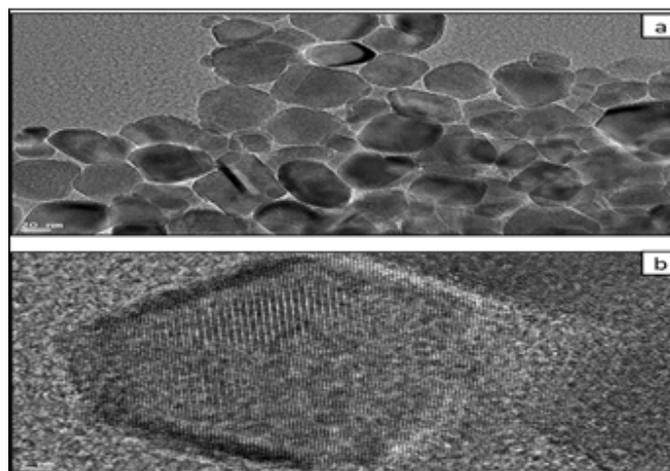


Figure 1: Showed characterization of prepared silver nanoparticles. The TEM micrograph of the aggregation of suspended silver nanoparticles in water used for intraperitoneal administration which ranging from $18\pm 5\text{nm}$ in diameter. HRTEM image showed the Ag NPs hexagonal in shape $18\pm 5\text{nm}$ diameter. a TEM electron micrograph of silver nanoparticles. (Bar = 20 nm). b HRTEM image of Ag nanoparticle hexagonal shape, scale bar 2 nm.

3.2 Kidney function tests

Table 1: Comparison of the effects of intraperitoneal injections of different concentrations of silver nanoparticles on serum creatinine levels (umol/L), uric acid (umol/L) and blood urea nitrogen (mmol/L) in adult male albino mice in at various weeks.

| Groups | 1 st week | | 2 nd week | | 4 th week | |
|-------------------------------------|----------------------|---------|----------------------|-------------------------------|----------------------|--------------------------------|
| | Mean ± SD | P-value | Mean ± SD | P-value | Mean ± SD | P-value |
| Creatinine levels (umol/L) | | | | | | |
| Control | 12.00±1.00 | | 12.33±0.58 | 0.423 | 12.00±0.00 | 1.000 |
| LD Ag-NPs | 10.67±0.58 | 0.050* | 9.67±0.58 | 0.0001* 0.225 | 9.33±0.58 | 0.001* 0.184 |
| MD Ag-NPs | 12.67±0.58 | 0.282 | 7.33±0.58 | 0.0001* 0.004 ⁺ | 9.67±0.58 | 0.002* 0.035 ⁺ |
| HD Ag-NPs | 7.67±0.58 | 0.0001* | 10.33±0.58 | 0.003* 0.015 ⁺ | 11.00±1.00 | 0.094 0.010 ⁺ |
| Uric acid level (umol/L) | | | | | | |
| Control | 110.00±0.00 | - | 111.00±1.00 | 0.051 | 110.33±0.58 | 0.052 |
| LD Ag-NPs | 201.67±3.51 | 0.0001* | 211.33±0.58 | 0.0001* 0.054 | 215.67±1.16 | 0.0001* 0.012 ⁺ |
| MD Ag-NPs | 245.33±4.04 | 0.0001* | 212.67±1.16 | 0.0001* 0.003 ⁺ | 216.33±1.53 | 0.0001* 0.003 ⁺ |
| HD Ag-NPs | 437.67±17.16 | 0.0001* | 271.67±3.51 | 0.0001* 0.003 ⁺ | 318.33±1.53 | 0.0001* 0.007 ⁺ |
| Blood urea nitrogen (mmol/L) | | | | | | |
| Control | 7.33±0.58 | | 7.00±0.00 | 0.423 | 7.00±1.00 | 0.742 |
| LD Ag-NPs | 8.33±0.58 | 0.122 | 10.33±0.58 | 0.0001* 0.074 | 10.33±0.58 | 0.001* 0.074 |
| MD Ag-NPs | 9.33±0.58 | 0.009* | 13.67±0.58 | 0.0001* 0.006 ⁺ | 14.33±0.58 | 0.0001* 0.01 3 ⁺ |
| HD Ag-NPs | 11.00±1.00 | 0.0001* | 17.00±1.00 | 0.0001* 1.000 | 17.00±1.00 | 0.0001* 1.000 |

Data are expressed as mean +/- standard deviation. *: significance versus control group using One-way ANOVA (LSD) test. +: significance versus 1st week in the same group using paired student "t" test.

The results showed that serum creatinine was significantly lowered in low, medium and high doses treated groups at 2nd and 4th tested weeks and in the low and high doses at 1st week versus control groups. Serum creatinine levels were significantly decreased in 2nd and 4th weeks in MD AgNPs treated group compared with 1st week. While, in HD Ag-NPs treated group, serum creatinine levels at 2nd and 4th weeks were significantly increased compared with 1st week. Serum level of uric acid (umol/L) after i.p. injection of Ag-NPs was significantly higher in LD AgNPs, MD AgNPs and HD AgNPs treated groups in the 1st, 2nd and 4th weeks compared with control. In LD AgNPs treated group, uric acid level was significantly increased in 4th compared to 1st week. While in MD AgNPs and HD AgNPs treated groups, uric acid levels were significantly decreased in the 2nd and 4th weeks after treatment compared with 1st week. At 2nd and 4th week of Ag-NPs administration, serum urea nitrogen was significantly increased in low, medium and high dose treated groups while, at the 1st week, it was significantly increased in medium and high dose treated groups compared with control. At 2nd and 4th week of Ag-NPs administration, serum urea nitrogen level was significantly higher in MD Ag-NPs treated groups compared with control.

3.3 Histopathological study of the kidney tubules and Corpuscles

In the 1st week, renal tubules in the control group showed normal tubules with narrow lumen, cuboidal epithelial lining having vesicular nuclei, capillaries around the tubules are non-congested. Renal tubules in LD group showed dilated Lumina, low height epithelium and the capillaries around tubules are congested. In the MD group, tubular section showed disorganization of tubule shape, dilation of tubule Lumina that contains some protein casts and congested capillaries. In the HD group, tubules showed marked dilation with reticulated content (Figure 2).

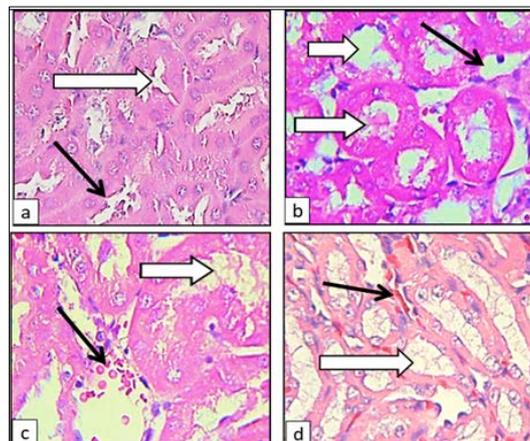


Figure 2: Sections from mice kidney in first week to show renal tubules of **a. control:** Normal tubules with narrow lumen (white arrow) cuboidal epithelial lining having vesicular nuclei (arrows). Capillaries around the tubules are non-congested (black arrow). **b. LD:** Renal tubules showed dilated lumina and low height epithelium (white arrow). Capillaries around tubules are congested (black arrow). **c. MD:** Disorganization of tubule shape and dilation of tubule lumina (white arrows) **some contains** protein casts. Capillary congestion could be seen (black arrow). **d. HD.** Showed marked dilated tubules with reticulated content (white arrow) (H & E stain x 400).

At the 1st week, the Bowman's capsule in the control group showed normal renal corpuscle and glomerulus. LD AgNPs group showed normal renal corpuscle with normal Bowman capsule and normal glomerulus. While, MD AgNPs group showed enlarged renal corpuscle, wide Bowman space, dilated glomerulus capillaries with decrease cellularity. HD AgNPs group showed normal renal corpuscle and normal glomerulus (Figure 3).

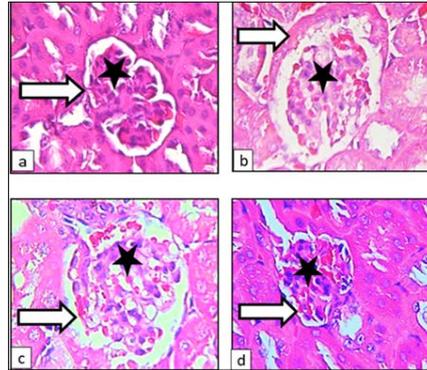


Figure 3: Sections in cortical regions of mice kidney in First week to show **a. control:** showing normal renal corpuscle (white arrow) and glomerulus (star). **b. LD:** Normal renal corpuscle with normal Bowman capsule (white arrow) and normal glomerulus (star). **c. MD:** enlarged renal corpuscle, wide Bowman space (white arrow), dilated glomerulus capillaries with decrease cellularity (star). **d. HD:** normal renal corpuscle (white arrow) and normal glomerulus (star) (H&E stain x1000).

In the 2nd week, microscopic examination of the tubules of the control group showed normal tubules with narrow lumen lined with cuboidal epithelial that had vesicular nuclei. The capillaries around the tubules are non-congested. In the LD group, tubules showed marked dilation with loss of outlines. The lumen contains fine reticulated material. Examination of MD group showed loss of tubule outlines, dilated lumen, and presence of reticulated materials. HD group showed marked tubule dilation that contains hyaline protein casts (Figure 4).

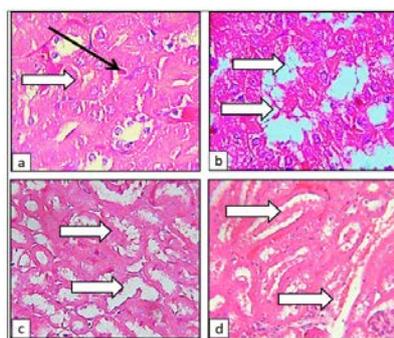


Figure 4: Sections from mice kidney in Second week to show renal tubules of **a. control:** normal tubules with narrow lumen (white arrow) cuboidal epithelial lining having vesicular nuclei (arrows). Capillaries around tubules are non-congested (black arrow). **b. LD:** marked dilation of tubules with loss of outlines. The lumen contains fine reticulated material (white arrows). **c. MD:** showed similar loss of tubule outline's dilation, some contain reticulated materials (arrows). **d. HD:** marked tubule dilation and most full of hyaline protein casts (white arrows). (H&E stain x 400)

The 2nd week showed that in the control group, normal renal corpuscle and glomerulus. In LD AgNPs group showed wide Bowman space and disorganization of glomerulus with capillary dilation. In MD AgNPs group showed also irregular Bowman capsule and wide space, glomerular dilation and decrease cellularity. HD AgNPs group showed wide capsular space and glomerular congestion (Figure 5).

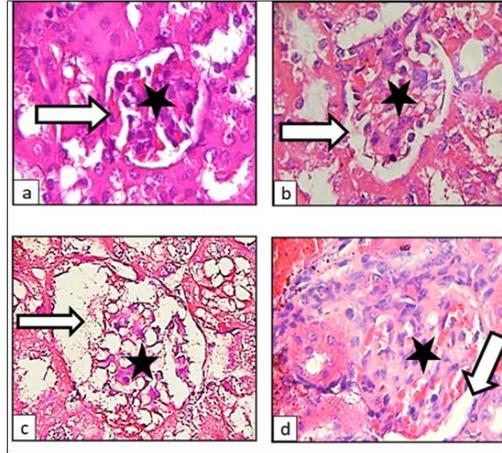


Figure 5: Sections in cortical regions of mice kidney in second week to show. **a. control:** showing normal renal corpuscle (white arrow) and glomerulus (star). **b. LD:** showed wide Bowman space (white arrow) and disorganization of glomerulus with capillary dilation (star). **c. MD:** showed also irregular Bowman capsule and wide space (white arrow), glomerular dilation and decrease cellularity (star). **d. HD:** showing wide capsular space (white arrow) and glomerular congestion (star). (H&E stain x1000)

In the fourth week, renal tubules of control group showed narrow lumina. Tubules in LD group showed increased of tubular dilation with presence of casts and marked capillary congestion. Renal tubules of MD group showed marked tubular dilation and presence of luminal reticulated material. Most tubules showed dilated lumina and presence of protein casts in HD treated group (Figure 6).

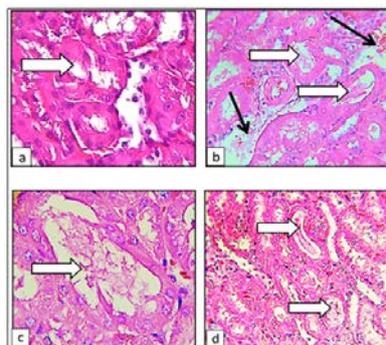


Figure 6: Sections from mice kidney in Fourth week to show renal tubules of a. Control: showing normal tubules with narrow lumina (white arrows). b. LD: showing increased of tubular dilation (arrows) with presence of casts. Marked capillary congestion (black arrows). c. MD: marked tubular dilation and presence of luminal reticulated material (white arrow). d. HD: most tubules showed dilated lumina and presence of protein casts. (H&E stain x 400)

The 4th week showed that in control group, normal renal corpuscle and Bowman capsule and normal glomerulus. In LD AgNPs group, irregular Bowman capsule with wide space and glomerular capillary congestion. In MD AgNPs group, irregular Bowman capsule with wide space and slight capillary congestion. In HD AgNPs group, marked decrease in renal corpuscle size with wide Bowman space and glomerular capillary congestion (Figure 7).

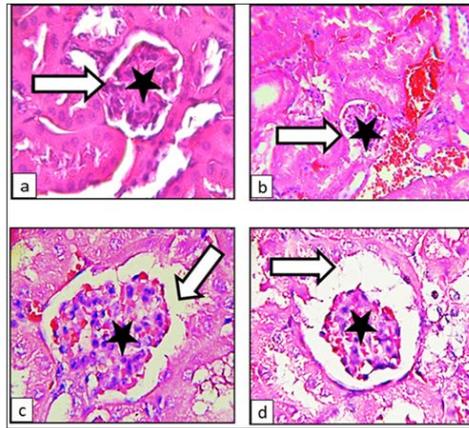


Figure 7: Sections in cortical regions of mice kidney in Fourth week to show **a. control:** normal renal corpuscle and Bowman capsule (white arrow) and normal glomerulus (star). **b. LD:** irregular Bowman capsule with wide space (white arrow) and glomerular capillary congestion (star). **c. MD:** irregular Bowman capsule with wide space (white arrow) and slight capillary congestion (star). **d. HD:** marked decrease in renal corpuscle size with wide Bowman space (white arrow) and glomerular capillary congestion (star) (H&E stain x1000).

4. Discussion

Silver nanoparticles are widely used in various fields as food, consumer, health care, and industrial purposes, due to their specific physical and chemical characters. These had electrical, optical, thermal, electrical conductivity and biological properties [20]. Khodadadi and his colleagues [21] stated that the silver nanoparticles have high toxicity than other materials. Silver nanoparticles can destruct different microorganisms, so, have side effects like many medications as after that entering the body, they are metabolized in liver and excreted by the kidneys, therefore, liver and kidney are affected more than other organs [22].

The properties of silver nanoparticles such as size, shape, surface charge, covering, agglomeration, and dissolving rate are important for their biological actions. Smaller particles have larger surface area and, so have a greater toxic potential than large particles [23]. The rate of particle dissolution depends on its chemical and surface properties as well as on its size and is affected by the surrounding media [24]. This process leads to surface oxidation and liberation of cytotoxic silver ions. High resolution transmission electron microscope images of Ag-NPs at various magnifications confirmed hexagonal shapes of silver nanoparticles with most clearly apparent average size of 18 ± 5 nm. HRTEM micrograph reveals many details at higher magnification with clearly observable crystallographic planes of silver. The repeat unit profile for interplanar distance is

around 0.24 nm. These results were in agreement with the author [25] who found that the lattice space profile Ag NPs is about 0.24nm. After studying the UV-Vis spectrum of silver nanoparticles, shown above, showed a hump at nearly 370 nm corresponding to band gap width of 3.35 e. v. which confirm the presence of silver nanoparticles. This band gap value may enhance the performance of silver nanoparticles for kidney toxicity male albino mice. These results come in accordance with those of Sastry and his colleagues [26] who found the band gap with of silver nanoparticle of 3.35 e. v.

The results of the present work showed that AgNPs cause alterations in the blood biochemical parameters. Blood urea nitrogen is a marker of kidney health. Creatinine is a waste product excreted by the kidneys. It accurately assesses the underlying kidney functions as it is less affected by diet type, stress and dehydration. Blood urea nitrogen and creatinine are two important factors to assess kidney functions and level of these factors in blood serum is increased by kidney destruction [27]. In the current study, it was showed the effects of intraperitoneal injections of low, medium and high doses of AgNPs on serum creatinine levels in different groups of adult male albino mice at 1st, 2nd and 4th weeks. The results showed that serum creatinine was significantly lowered in low, medium and high doses treated groups at 2nd and 4th tested weeks and in the low and high dose at 1st week versus control groups. The result of the experimental studies by the author [28] demonstrated that Ag-NPs affected the kidney functions in gender-dependent manner so that marked kidney toxicity was reported in female mice. This was indicated by a significant decrease of serum creatinine level and hyperkalemia in female mice than male. On the other hand, Jarrar and his colleagues [29] found that the creatinine blood levels were raised significantly in all mice administered AgNPs in comparison with control ones. The results of current work showed that the serum urea nitrogen and uric acid were significantly increased in low, medium and high dose treated groups while, at the 1st week, it was significantly increased in medium and high dose treated groups than control. These results are accordance with Abdel-Raouf and his colleagues [30] who indicated that blood urea nitrogen, uric acid and creatinine activities were significant elevated in gentamicin intoxicated rats in comparison to control group. On the contrary, Jarrar and his colleagues [29] mentioned nonsignificant elevation of uric acid level in the blood of mice received 10 nm silver particles. Heydrnejad and Samani [28] reported that the administration of Ag-NPs to male and female mice produced changes in serum levels of blood urea nitrogen and creatinine.

Many studies revealed that exposure of AgNPs led to clear accumulation in different organs as liver, kidneys, testes, lungs, and brain [7, 31, 32]. Previously, the liver and kidneys have been described as main organs for silver distribution, whether the exposure was orally [31], intravenously [33], subcutaneously [8] or through inhalation [34]. In accordance with the previous results, the present study proved that the histopathological findings of the kidney male albino mice after single intraperitoneal administrated with AgNPs through 1st, 2nd and 4th weeks were affected male albino mice kidney histology represented by necrosis, cytoplasmic vacuoles, dilation and congestion of central vein, In kidney there are some histological changes such as irregular in renal corpuscle size, wide Bowman space, dilated glomerulus capillaries with decrease cellularity and glomerular capillary congestion. Disorganization of tubule shape and dilation of tubule Lumina some contains protein casts. *In vivo* studies have shown that AgNPs are related to injuries to the brain, liver, and lung [35]. In the kidneys, accumulations have been reported to occur in renal glomerular basement membrane [36, 37] and in the mesangium [38]. The histological view in treated kidney's tissue with Ag NPs demonstrated necrosis of

glomerular cells, bowman capsule and proximal tubular region with program cell death sequences [39]. Previous studies on experimental animals and *in vitro* have reported that toxicity of silver nanoparticles is related to oxidative stress induction [40], apoptosis [40] and induction of lipid peroxidation [41]. The effects of silver nanoparticle on the kidney were that the cytoplasm showed some mitochondria with destroyed cristae and numerous large membranous vesicles. The glomeruli showed podocytes with swollen and elongated primary and secondary processes, and the basement membrane of the endothelial cells in the capillary tufts was thickened [42]. Other investigators have reported deposition of ingested silver in the renal tubules and glomerular basement membrane [43], as well as mesangial cells proliferation [44]. Further, swelling of renal epithelium and presence of membranous vacuoles along with hypertrophied nucleoli had been shown in animals treated with AgNPs [45, 46, 47].

5. Conclusions

We concluded that the organs mostly affected after intraperitoneal injection of AgNPs were the kidneys. AgNPs can induce several morphological and functions alterations of the kidneys that is dose and time dependent that leads to destruction of the glomeruli and tubules of the kidneys. Further studies of AgNPs are being conducted in mice for a better understanding of the pathway mechanisms underlying those lesions

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References

- [1]. S. Kim, D.Y. Ryu. "Silver nanoparticle-induced oxidative stress, genotoxicity and apoptosis in cultured cells and animal tissues." *Journal of Applied Toxicology*, vol. 33(2), pp.78-9, 2013.
- [2]. I. Sondi, B. Salopek-Sondi. "Silver nanoparticles as antimicrobial agent: a case study on E. coli as a model for Gram-negative bacteria." *J Colloid Interface Sci*, vol. 275(1), pp.177-82, 2004.
- [3]. A. Panacek, M. Kolar, R. Vecerova, R. Prucek, J. Soukupova, V. Krystof, et al. "Antifungal activity of silver nanoparticles against *Candida* spp." *Biomaterials*, vol. 30 (31), pp.6333- 40, 2009 .
- [4]. R.W. Sun, R. Chen, N.P. Chung, C.M. Ho, C.L. Lin, C.M. Che. "Silver nanoparticles fabricated in HEPES buffer exhibit cytoprotective activities toward HIV-1 infected cells." *Chem Commun (Camb)*, vol. 40, pp.5059-5061, 2005.
- [5]. C. Graf, D.L. Vossen, A. Imhof, A. Van Blaaderen. "A general method to coat colloidal particles with silica." *Langmuir*, vol. 19 (17), pp.6693-6700, 2003.
- [6]. J. Tang, T. Xi. "Status of biological evaluation on silver nanoparticles." *Journal of biomedical engineering*, vol. 25, pp. 958-961, 2008.
- [7]. S. Kim, J.E. Choi, J. Choi, K.H. Chung, K. Park, J. Yi, et al. Oxidative stress-dependent toxicity of silver nanoparticles in human hepatoma cells. *Toxicology in vitro*, vol. 23(6), pp.1076-1084, 2009.
- [8]. J. Tang, L. Xiong, S. Wang, J. Wang, L. Liu, J. Li, et al. "Distribution, translocation and accumulation

- of silver nanoparticles in rats.” *Journal of nanoscience and nanotechnology*, vol. 9(8), pp.4924-4932, 2009.
- [9]. F. Faedmaleki, F.H. Shirazi, A.A. Salarian, A.H. Ashtiani, H. Rastegar. “Toxicity Effect of Silver Nanoparticles on Mice Liver Primary Cell Culture and HepG2 Cell Line.” *Ira J Pharm Res*, vol. 13 (1), pp.235-242, 2014.
- [10]. A. Rinna, Z. Magdolenova, A. Hudecova, M. Kruszewski, M. Refsnes, M. Dusinska. “Effect of silver nanoparticles on mitogen-activated protein kinases activation: role of reactive oxygen species and implication in DNA damage.” *Mutagenesis*, vol. 30 (1), pp.59-66, 2015.
- [11]. J.S. Teodoro, A.M. Simoes, F.V. Duarte, A.P. Rolo, R.C. Murdoch, S.M. Hussain, et al. “Assessment of the toxicity of silver nanoparticles in vitro: a mitochondrial perspective.” *Toxicol In Vitro*, vol. 25(3), pp.664-670, 2011.
- [12]. F. Oesch, R. Landsiedel. “Genotoxicity investigations on nanomaterials.” *Archives of toxicology*, vol. 86(7), pp.985-994, 2012.
- [13]. Y.S. Kim, M.Y. Song, J.D. Park, K.S. Song, H.R. Ryu, Y.H. Chung, et al. “Subchronic oral toxicity of silver nanoparticles.” *Particle and fibre toxicology*, vol. 7, pp.20, 2010a.
- [14]. V.S. Ramkumar, A. Pugazhendhi, K. Gopalakrishnan, P. Sivagurunathan, G.D. Saratale, T.N.B. Dung, et al. “Biofabrication and characterization of silver nanoparticles using aqueous extract of seaweed *Enteromorpha compressa* and its biomedical properties.” *Biotechnology reports*, vol. 14: pp.1-7, 2017.
- [15]. M.A. Ansari, H.M. Khan, A.A. Khan, M.A. Alzohairy, M. Waseem, M.K. Ahmad, et al. “Biochemical, histopathological, and transmission electron microscopic ultrastructural changes in mice after exposure to silver nanoparticles.” *Environmental toxicology*, vol. 31(8), pp.945-956, 2016.
- [16]. K. Jung. “Tietz Fundamentals of Clinical Chemistry”, 6th edition. Carl A. Burtis, Edward R. Ashwood, David E. Bruns, editors. St Louis, MO: Saunders/Elsevier, pp.976, 2008.
- [17]. H.A. Bulger, H.E. Johns. “The determination of plasma uric acid.” *Journal of Biological Chemistry*, vol. 140, pp.427-440, 1941.
- [18]. H.M. Kalckar, M. Shafran, M. “Differential spectrophotometry of purine compounds by means of specific enzymes. 3. Studies of the enzymes of purine metabolism.” *Journal of Biological Chemistry*, vol. 167, pp.461-475, 1947.
- [19]. R.A.B. Drury, E.A. Wallington. “Preparation and fixation of tissues.” *Carleton's histological technique*, vol. 5, pp.41-54, 1980.
- [20]. C.L. Keat, A. Aziz, A.M. Eid, N.A. Elmarzugi. “Biosynthesis of nanoparticles and silver nanoparticles.” *Bioresources and Bioprocessing*, vol. 2, pp.47, 2015.
- [21]. B. Khodadadi, M. Bordbar, M. Nasrollahzadeh. “*Achillea millefolium* L. extract mediated green synthesis of waste peach kernel shell supported silver nanoparticles: Application of the nanoparticles for catalytic reduction of a variety of dyes in water.” *J Colloid Interface Sci.*; vol. 493, pp.85-93, 2017.
- [22]. S. Moaddab, H. Ahari, D. Shahbazzadeh, A.A. Motallebi, A.A. Anvar, J. Rahman-Nya, et al. “Toxicity study of nanosilver (nanocid?) on osteoblast cancer cell line.” *International Nano Letters*, vol. 1, pp.11, 2011.
- [23]. H.J. Johnston, G. Hutchison, F.M. Christensen, S. Peters, S. Hankin, V. Stone. “A review of the in vivo

- and in vitro toxicity of silver and gold particulates: particle attributes and biological mechanisms responsible for the observed toxicity.” *Critical reviews in toxicology*, vol. 40, pp. 328-346, 2010.
- [24]. S.K. Misra, A. Dybowska, D. Berhanu, S.N. Luoma, E. Valsami-Jones. “The complexity of nanoparticle dissolution and its importance in nanotoxicological studies.” *Science of the total environment*, vol. 438, pp.225-232, 2012.
- [25]. M.F. Anwar, D. Yadav, S. Kapoor, J. Chander, M. Samim, M. “Comparison of antibacterial activity of Ag nanoparticles synthesized from leaf extract of *Parthenium hysterophorus* L in aqueous media and gentamicin sulphate: in-vitro.” *Drug development and industrial pharmacy*, vol. 41, pp.43-50, 2015.
- [26]. J. Sastry, P. Bakas, H. Kim, L. Wang, A. Marinopoulos. “Evaluation of cascaded H-bridge inverter for utility-scale photovoltaic systems.” *Renewable Energy*, vol. 69, pp.208-218, 2014.
- [27]. O. Dayani, A. Ghiasi, R. Tahmasbi. “Chemical Composition, Physical Characteristics, Digestibility and Degradability of Grape Pomace Processed with *Neurospora sitophila*.” *Iranian Journal of Applied Animal Science*, vol. 4(4), pp.733-739, 2014.
- [28]. M.S. Heydrnejad, R.J. Samani. “Sex Differential Influence of Acute Orally-administered Silver nanoparticles (Ag-NPs) on Some Biochemical Parameters in Kidney of Mice *Mus musculus*.” *J Nanomed Nanotechnol*, vol. 7(3), pp.1-5, 2016.
- [29]. Q. Jarrar, A. Battah, F. Obeidat, K. Battah. “Biochemical changes induced by the toxicity of variable sizes of silver nanoparticles.” *British Journal of Pharmaceutical Research*, vol. 4(24), pp.2670-2678, 2014.
- [30]. N. Abdel-Raouf, N.M. Al-Enazi, I.B.M. Ibraheem, R.M. Alharbi, M.M. Alkhulaifi. “Bactericidal efficacy of Ag and Au nanoparticles synthesized by the marine alga *Laurencia catarinensis*.” *International Journal of Pharmaceutical Research & Allied Sciences*, vol. 6(2), pp.213-226, 2017.
- [31]. E.J. Park, E. Bae, J. Yi, Y. Kim, K. Choi, S.H. Lee, et al. “Repeated-dose toxicity and inflammatory responses in mice by oral administration of silver nanoparticles.” *Environmental toxicology and pharmacology*, vol. 30, pp.162-168, 2010.
- [32]. F. Li, M.D. Weir, J. Chen, H.H. Xu, H.H. “Comparison of quaternary ammonium-containing with nano-silver-containing adhesive in antibacterial properties and cytotoxicity.” *Dental Materials*, vol. 29, pp.450-461, 2013.
- [33]. K. Park, E.J. Park, I.K. Chun, K. Choi, S.H. Lee, J. Yoon, B.C. Lee, et al. “Bioavailability and toxicokinetics of citrate-coated silver nanoparticles in rats.” *Archives of pharmacal research*, vol. 34, pp.153-158, 2011.
- [34]. Takenaka, S., Karg, E., Roth, C., Schulz, H., Ziesenis, A., Heinzmann, U., Schramel, P. and Heyder, J., 2001. Pulmonary and systemic distribution of inhaled ultrafine silver particles in rats. *Environmental health perspectives*, vol. 109, pp.547. 2001.
- [35]. H.S. Sharma, S. Hussain, J. Schlager, S.F. Ali, A. Sharma. “Influence of nanoparticles on blood–brain barrier permeability and brain edema formation in rats.” *Acta Neocortex Suppl*, vol. 106, pp.359-364, 2010.
- [36]. D. Moffat, M. Creasey. “The distribution of ingested silver in the kidney of the rat and of the rabbit.” *Cells Tissues Organs*, vol. 83, pp.346-355, 1972.
- [37]. G. Danscher. “Light and electron microscopic localization of silver in biological tissue.”

- Histochemistry, vol. 71, pp.177-186, 1981.
- [38]. W. Day, J. Hunt, A. Mcgiven. "Silver deposition in mouse glomeruli." *Pathology*, vol. 8, pp. 201-204. 1976.
- [39]. J.P. Pani, R. Singh. "Small Size Nanosilver Multi Organ Toxicity: A Higher Dose Negative Response in In-Vivo and In-Vitro Experimental Application, Department of Anatomy." *Biomed J Sci & Tech Res*, vol. 1(4), pp.1-6, 2017.
- [40]. A.M. Schrand, L.K. Braydich-Stolle, J.J. Schlager, L. Dai, S.M. Hussain. "Can silver nanoparticles be useful as potential biological labels?" *Nanotechnology*, vol. 19, pp. 235104, 2008.
- [41]. S. Arora, J. Jain, J. Rajwade, K. Paknikar. "Interactions of silver nanoparticles with primary mouse fibroblasts and liver cells." *Toxicology and applied pharmacology*, vol. 236, pp.310-318, 2009.
- [42]. O.M.M. Sarhan, R.M. Hussein. "Effects of intraperitoneally injected silver nanoparticles on histological structures and blood parameters in the albino rat." *International journal of nanomedicine*, vol. 9, pp.1505, 2014.
- [43]. Y.J. Kim, S.I. Yang, J.C. Ryu. "Cytotoxicity and genotoxicity of nano-silver in mammalian cell lines." *Molecular & Cellular Toxicology*, vol. 6, pp.119-125, 2010.
- [44]. G. Terentyuk, G. Maslyakova, L. Suleymanova, V. Borodulin, Y.S. Dudakova, N. Khlebtsov, et al. "Morphological and biochemical changes after intravenous injection of gold nanoparticles." *Photonics and Optoelectronics Meetings, International Society for Optics and Photonics, Seventh International Conference on Photonics and Imaging in Biology and Medicine; 2009, China*.
- [45]. M.J. Kogan, N.G. Bastus, R. Amigo, D. Grillo-Bosch, E. Araya, A. Turiel, et al. "Nanoparticle-mediated local and remote manipulation of protein aggregation." *Nano letters*, vol. 6, pp.110-115, 2006.
- [46]. M. Noginov, G. Zhu, M. Bahoura, J. Adegoke, C.Small, B. Ritzo, et al. The effect of gain and absorption on surface plasmons in metal nanoparticles. *Applied Physics B: Lasers and Optics*, vol. 86, pp. 455-460, 2007.
- [47]. J. Yousef, H. Hendi, F.S. Hakami, M.A. Awad, A.F. Alem, A.A. Hendi, et al. "Toxicity of silver nanoparticles after Injected intraperitoneally in rats," *J Am Sci*, vol. 8, pp.589-593, 2012.