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Investigating the influence of biosynthetic silver nanoparticles on kidney and tissue function in mice

Nabaa Abbas Zubaidi[1,*], *Mustafa Kareem AL-Azzawi*[1], *Marwan Saleh Mahdi*[1], *Murtadha Hadi Ajmi*[2]

[1]Department of Biology, College of Education for Pure Sciences, University of Wasit, Iraq
[2]Department of Biology, College of Sciences, University of Wasit, Iraq
[*]) Email: mqasim@uowasit.edu.iq

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The standard of living and supply innovative nanoparticles solutions to the tests of the present. Owing to their unique properties, such as high conductivity and antibacterial activity, silver nanoparticles (AgNPs) have developed one of the most widely used nanomaterials. Anxieties regarding AgNPs' potential damaging properties on ecosystems and living organisms persevere, nonetheless their benefits. The purpose of this study is to improvement a better understanding of the physiological and histological properties of AgNPs on white Swiss mice by investigative the effects of exposure on behavior organ function, and tissue structure. Staphylococcus aureus strains are used for the creation of AgNPs. For seven and fourteen days, respectively there are two groups of participants are given intraperitoneal injections of 150 mg/kg AgNPs the two groups serving as controls are given sodium chloride solution for the same period of time. Each of the four categories is allocated a different set of twenty-four mice. The physiological part analyses included testing blood samples for urea, creatinine, and uric acid. Histological analysis is done on kidney tissues to notice the structural changes. Mice injected with AgNPs exhibited a loss of strength, decreased hunger and decreased body weight. Blood urea and creatinine levels are significantly higher in the experimental groups compared to the controls. Histological analysis exposed substantial changes in the kidney tissues including a shrinkage of the glomeruli and an expansion of Bowman's capsule gap these marks of inflammation and necrosis. The best result that Mice injected with 150 mg/kg Ag NPs displayed increased lethargy, introversion, back hunching, and appetite loss. Weight measurements showed significant weight loss after 14 days in the Ag NPs group compared to controls. Physiologically, there are significant increases in urea and creatinine levels in the Ag NPs group after 7 and 14 days, indicating potential kidney damage. Histological analysis revealed a decrease in glomerular diameter and an increase in Bowman's capsule space in the Ag NPs group, further supporting the presence of nephrotoxicity. The study aims to examine the impact of biosynthetic silver nanoparticles on the kidney and tissue function in white Swiss mice. Exactly, the study emphases on investigative phenotypic, behavioral, physiological, and histological

Exp. Theo. NANOTECHNOLOGY 9 (2025) 15-26
changes in mice following exposure to silver nanoparticles, to evaluate potential nephrotoxicity and general health risks related with nanoparticle exposure.

Keywords: AgNPs; Kidney function; Histological analysis; Urea.

1. INTRODUCTION

Quickly emerging nanotechnology produces a likelihood for obtaining a huge spectrum of nanoparticles (NPs) of diverse composition, shape and size on an industrial scale. Due to their biological, chemical physical characteristics, which are specified by their separate optical and physical properties, in addition to biochemical functionality, NPs present their application not alone in the manufacturing, but moreover in medicine [1]. Nanotechnology has generated attention in many fields like healthcare, manufacturing and agriculture in recent times. This is mostly due to its potential to improve quality of life and suggestion solutions to challenges [2]. Among the applications, silver nanoparticles (AgNPs) stand out as one of the most widely used nanomaterials due to their unique properties such as antibacterial effects due to their surface reactivity and excellent conductivity. Biosynthesis of silver nanoparticles is preferred over chemical and physical method in medical applications because they are environmentally friendly, low toxicity and cost while chemical and physical methods are toxic, ineffective and high cost [3]. NPs can use their effects by a diversity of mechanisms, for example either indirect or direct interaction with the cell wall, biopolymers and intracellular compartments; initiation of intracellular effects, for instance the obstetrics of free radicals, nitrogen species, and reactive oxygen; and eliciting innate and adaptive host immune responses [4].

Recently, several studies have been conducted to explore the possibility of manufacturing silver nanoparticles using microorganisms as a potential biosource. Silver nanoparticles are manufactured using microorganisms or the overlying solution of microbial culture at rates much slower than those at which plants can manufacture silver nanoparticles. The time required for complete reduction of silver ions is known to range from 24 hours to 120 hours in the case of microorganisms, while the time required for the completion of the reaction is much less in the case of plants, ranging from a few hours to a maximum of 48 hours [5].

Research, on nanoparticles, like copper oxide (CuO) also yielded findings. Zinc oxide (ZnO-NPs), and titanium dioxide (TiO2-NPs) have demonstrated that exposure to nanoparticles impacts the structure and function of renal tissues. Histological analysis revealed that TiO2-NPs induced acute nephrotoxicity, characterized by dilated tubules and cell desquamation in the proximal tubules [6]. In vitro and in vivo, ZnO-NPs cause oxidative stress, mitochondrial damage, and death in renal tubular epithelial cells [7,8]. Ultrastructural damage in renal tissues by CuO nanoparticles includes mitochondrial enlargement, apoptotic activity, and nuclear abnormalities, suggesting renal tissue pathophysiology [9].

2. MATERIALS AND METHODS

2.1 Biosynthesis of Ag NPs

The *S. aureus* strains multiplied and are cultured in Mueller Hinton broth for 24 hours at 37°C. After that, the culture underwent centrifugation at 12,000 revolutions per minute aimed at 5 minutes. The resulting liquid above the sediment identified as the supernatant the is used for the production of silver nanoparticles (AgNPs). A total of 7 milliliters of Milli Q water is utilized as the solvent for the production of AgNPs [6]. The liquid portion is introduced individually into the reaction container that already contained silver (Ag) at a concentration of 10−3 (1% v/v). The supernatant is reacted with Ag+ ions under light circumstances for a duration of 5 minutes. The decrease of Ag+ ions in the solution is seen

and the spectra are measured using an ultraviolet-visible (UV-vis) spectrophotometer with a resolution of 1 nm. The AgNPs are studied using scanning electron microscopy (SEM) [10].

2.2 Preparation Nanomaterial Concentration and Nanoparticle Characterization

750 mg of Nano silver is dissolved in 20 ml of 0.5% sodium chloride saline solution, formerly it is positioned in the mechanical stirrer for 30 minutes for the determination of mixing the material well, and then placed in the Sonicator Ultrasonic Liquid Processor for 15 minutes to confirm a homogeneous compound and get rid of agglomerates, after which the laboratory animal is injected with 0.1 ml using an insulin syringe into the peritoneal cavity today and during two time periods 7 and 14 days. Identification of silver nano particle in Nano-center (Shahid chamran University of Ahvaz in Iran) by using the following methods; Scanning Electron Microscopy (SEM) [11] and UV-VIS Spectroscopy in the range of (200-800) nm, The SPR peak's position in the UV-VIS spectrum (typically around 400-450 nm for silver nanoparticles) helps identify the presence and nature of the nanoparticles [12].

3. EXPERIMENTAL DESIGN

The effects of sodium chloride and silver nanoparticles on white Swiss mice are the topics of an experimental investigation. There are twenty-four mice total with six animals spread across four groups. The first group of mice underwent a seven-day course by injections intraperitoneal of 0.1 ml of a solution containing 150 mg/kg of silver nanoparticles. The second group of mice are given the equal volume and concentration for a period of 14 days. The third group got injections of a 0.9% sodium chloride solution for seven days, and the fourteen days, the same solution is given to the fourth group. The investigators kept close tabs on the mice throughout the experiment that noting any variations in their behavior or appearance. The Mice are partially anesthetized with chloroform and blood samples are taken after the injection period In order to isolate the serum, then samples are centrifuged after being placed in tubes with a gel.

4. Physiological and Histological Measurements

4.1 Urea Measurement

10 µL of serum is mixed with 1000 µL of Reagent 1 (R1) from the Spinreact kit and incubated at 37°C for 5 minutes. Then, 1000 µL of Reagent 2 (R2) is added and incubated for another 5 minutes. Absorbance is measured at 590 nm using a spectrophotometer.

4.2 Creatinine Measurement

10 µL of serum is mixed with 1000 µL of the reagent and incubated for 5 minutes. Absorbance is measured at 490 nm initially (A1) and after 90 seconds (A2). Creatinine concentration is determined from the difference (A1 - A2).

4.3 Uric Acid Measurement

20 µL of serum, standard solution, and blank solution are each mixed with 1000 µL of Reagent 1 (R1) and incubated at 37°C for 5 minutes. Absorbance is measured at 520 nm using a UV-Vis spectrophotometer.

4.4 Tissue Preparation

Tissues are stabilized with formaldehyde solution, dehydrated through a graded alcohol series, cleared in xylene, and embedded in paraffin wax. Sections are cut at 5 μm thickness, stained with hematoxylin and eosin, mounted on slides with DPX, and examined under a light microscope. Histological slides are examined using a compound light microscope equipped with an imaging camera. Histological changes are evaluated. Images of histological sections are captured using a digital camera connected to a light microscope. Measurements are made using Image J software to calculate the mean diameter of glomeruli and renal tubules.

4.5 Statistical Analysis

Data are analyzed using SPSS software to identify statistical differences between groups using T-tests and ANOVA. Results are presented as mean ± standard error.

5. RESULTS AND DISCUSSION

5.1 Characterization of Ag NP

The spectrum has a distinct absorption peak, as indicated by the orange curve. The primary absorption peak occurs at around 420 nm. Resonance refers to the phenomenon known as localized surface plasmon resonance (LSPR). The peak's high absorption also suggests a significant concentration of silver nanoparticles. The morphology and spatial orientation of the apex can provide insights into the dimensions and dispersion of particles inside the solution. Subsequently, the absorbance exhibits a significant decline following the peak at 420 nm, suggesting that the nanoparticles possess a highly homogeneous size distribution. For wavelengths less than 300 nm and greater than 500 nm, the absorptivity is minimal, indicating that silver nanoparticles mostly respond at wavelengths approximately 420 nm. Therefore, we can infer that the prominent peak at 420 nm signifies the successful synthesis of silver nanoparticles and their possession of distinct plasmonic characteristics [13]. These features have versatile applications, including antibacterial, medicinal, and photocatalytic uses. To verify the presence of nanoparticles, the absorption distribution is examined. A rather uniform size distribution suggests that the fabrication of nanoparticles is of excellent quality.

5.2 Measurement of UV-Vis Spectrum

The UV-Vis spectrum is measured using a solution of synthesized silver nanoparticles. A sample of the nanoparticle solution is placed in a quartz cuvette, and the absorbance is measured over a range of wavelengths from 300 nm to 800 nm using a UV-Vis spectrophotometer.

Exp. Theo. NANOTECHNOLOGY 9 (2025) 15-26

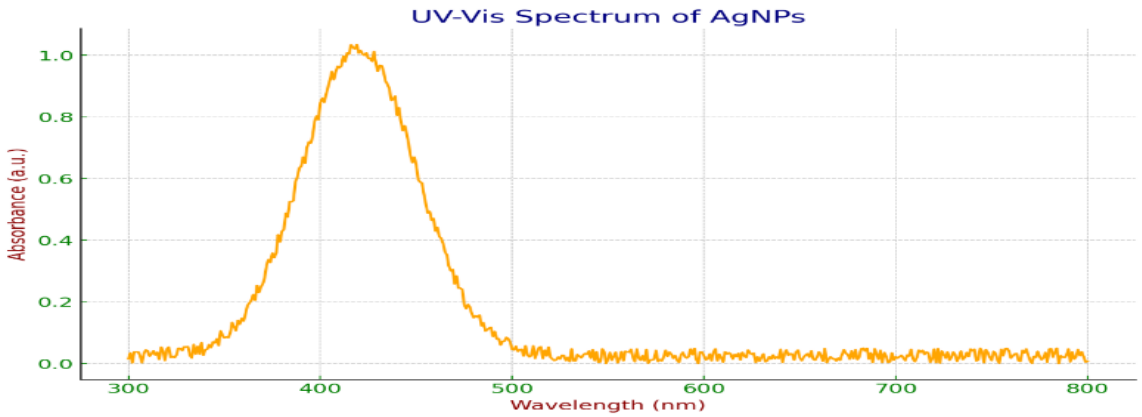

Figure 1 UV-Vis Absorption Spectrum Indicating LSPR of Synthesized Silver Nanoparticles at 420 nm.

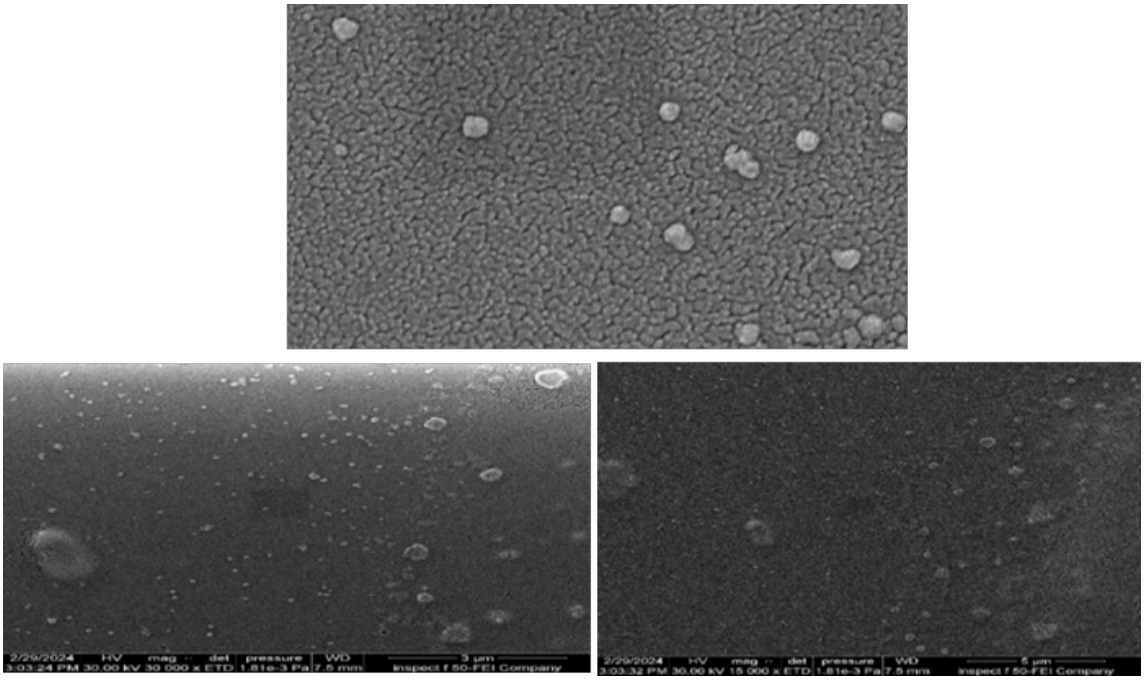

Figure 2 SEM Image of Synthesized Silver Nanoparticles Showing Spherical Morphology with Sizes Ranging from 45 to 97 nm.

Analysis of the AgNPs the morphological synthesis show AgNPs studied by SEM particles being mostly spherical in shape with average size is 45 to 97 nm show in figure 2. The SEM analysis shows that the synthesized Ag-NPs are spherical in shape. This study found phenotypic, behavioral, functional, and histological abnormalities in silver nanoparticle-injected mice. Lethargy, introversion, loss of appetite, and back hunching increased over time, indicating that injections affected the mice's overall health. Animals changed from greenish-brown to greenish-black in animals injected for 7 to 14 days. Histological changes included changes in the way the kidneys looked on the outside, as shown in Figure3 by the normal cannulated red color with fatty materials in the control group. On the other hand, silver nanoparticle-injected animals' kidneys are paler and lacked surrounding fatty content, especially

Exp. Theo. NANOTECHNOLOGY 9 (2025) 15-26
after 14 days Figure3. These findings imply that silver nanoparticles may negatively impact mice's behavior, appearance, organ functions, and histology, such as the kidneys, male mice injected with 150 mg/kg secondary Ag nanoparticles into the peritoneal cavity for seven days displayed lethargy, introversion, hunching back, and appetite reduction. With a 14-day injection, symptoms increased, and animal body weights fell in comparison to the control group. Male mice treated with high concentrations of silver nanoparticles (Ag NPs) showed neurotoxic behavioral changes [14]. Lethargy, introversion, hunching back, and appetite loss intensify with extended AgNP exposure. Ag NPs have a systemic effect on mice's physiological health, as evidenced by their reduced body weight compared to the control group. These symptoms might be caused by too much Ag NP, which is a good reminder that we need to look into how nanomaterial exposure might hurt living things [14].

The results of present study align with a recent study [15]. The accumulation of nanoparticles in the gastrointestinal tract affects food intake and digestion, causing animals to lose weight and fat around their organs (15). Aluminum oxide nanoparticles (AlNPs) lowered diet and water consumption, resulting in a reduced body weight increase in treated groups, suggesting digestive problems [15]. Furthermore, [16] showed that AgNPs lowered testes and epididymitis weights and affected sperm parameters in male albino mice. Histopathological results from different studies suggest that the change in kidney color to pale red after seven and fourteen days in animals injected with Ag nanoparticles compared to the control group may be due to renal glomeruli getting smaller and renal tissue not getting enough blood. Silver nanoparticles have been shown to damage kidney tissue, lead to vacuolar degeneration, and inflammatory cell aggregation, all of which affect the structure and function [17]. Copper nanoparticles have been linked to changes in the microstructure of kidney tissues. This shows how important antioxidant vitamins are in reducing the damage nanoparticles cause and restoring cell health [18]. The structure of the kidneys, including the diameters of the glomeruli and tubules, is also changed by zinc oxide nanoparticles, which suggests that they have toxic effects on kidney function [19].

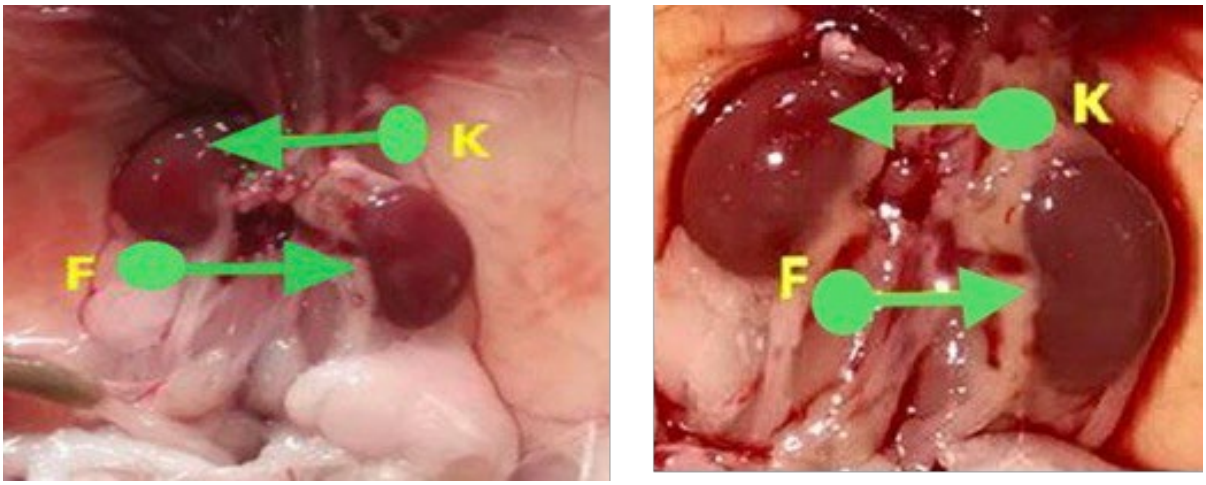

Figure 3 Kidneys and Related Structures in a Dissected Specimen.

The results showed a significant decrease in the average weights of animals injected intraperitoneal with a concentration of 150 mg/kg of silver nanoparticles for 14 days, where weights are recorded at an average of 23.52 grams compared to the control group, which recorded an average of 27.08 grams. In contrast, the seven-day duration of the injection did not show any significant difference in the average weights of the animals when compared with the control group, although there is a slight weight loss when compared with the same group before the injection, as shown in Table (1).

Exp. Theo. NANOTECHNOLOGY 9 (2025) 15-26

Table 1 Changes in Body Weights of All Experimental Animals before and After Injection.

Injection Duration (days)	Group	Body Weight Before Injection (g) ± SE	Body Weight After Injection (g) ± SE	Kidney Weight (mg) ± SE	Height (mm) ± SE	Width (mm) ± SE	Length (mm) ± SE
7	Control	29.35 ± 0.28	29.75 ± 0.68	0.23 ± 0.02	0.50 ± 0.00	0.65 ± 0.03	1.15 ± 0.03
7	150 mg/kg Ag NPs	31.02 ± 2.10	29.95 ± 1.68	0.24 ± 0.02	0.52 ± 0.02	0.62 ± 0.02	1.12 ± 0.04
14	Control	24.08 ± 0.73	27.08 ± 0.81	0.23 ± 0.02	0.53 ± 0.03	0.65 ± 0.03	1.13 ± 0.03
14	150 mg/kg Ag NPs	24.06 ± 0.45	23.52 ± 0.64	0.22 ± 0.01	0.50 ± 0.00	0.60 ± 0.00	1.11 ± 0.01

Values are presented as mean ± standard error (SE). Letters next to values indicate statistical significance; identical letters within the same column indicate no significant differences between groups ($P > 0.05$), while different letters indicate significant differences ($P \leq 0.05$) The study did not record any statistically significant differences in the weights and dimensions of the kidneys in mice injected with 150 mg/kg of silver nanoparticles for both 7 and 14 days compared to the control groups. Identical letters vertically indicate no significant differences between readings ($P > 0.05$), representing the mean ± standard error. Similar to the weights, identical letters vertically show no significant differences between measurements ($P > 0.05$), representing the mean ± standard error. These tables provide data on the absence of significant changes in kidney weights and dimensions post-injection with nanoparticles.

5.3 Physiological Study

The current study show experiential an increase in urine and creatinine levels. The Animals injected with 150 mg/kg of Ag nanoparticles into the cavity as well as civilians examined seven and 14 days later did not show a distinguished difference in uric acid levels compared to the control group. This absence of disparity could potentially explanation for the creatinine levels. The presence of Ag nanoparticles may chief to increased urine and creatinine levels within eight days after injection while blood serum uric acid concentrations might reduction during this period [17]. The exposure to doses of silver nanoparticles is related to urine creatinine levels after eight days. Moreover, exposure to AgNPs is linked with decreased blood serum acid levels [20]. Studies on adult male albino mice exposed to AgNP revealed effects on function resulting in elevated levels of creatinine, uric acid and blood urea nitrogen. Also kidney tissues exhibited variations such as localized necrosis and programmed cell death [20]. Remarkably, a study on the influences of AgNPs on female albino mice highlighted changes in tissues together with increased blood urea nitrogen and serum creatinine levels. The presence of stress markers indicating the generation of oxygen species is also renowned [17]. Once seeing all these findings, it raises questions, about the kidneys capacity to remove quantities of AgNPs. This could potentially chief to variations, in both blood acid levels and urine creatinine levels.

Exp. Theo. NANOTECHNOLOGY 9 (2025) 15-26

Table 2 Effect of Silver Nanoparticles (Ag NPs) on Renal Function Limitations in mice Over Altered Injection Times.

Group	Injection Duration (Days)	Urea Concentration (mg/dL) ± SE	Creatinine Concentration (mg/dL) ± SE	Uric Acid Concentration (mg/dL) ± SE
Control	7	36.00 ± 2.04a	0.88 ± 0.11a	4.10 ± 0.31a
Ag NPs 150 mg/kg	7	41.50 ± 1.84b	1.45 ± 0.19b	5.37 ± 1.10a
Control	14	37.25 ± 1.25a	0.92 ± 0.12a	3.88 ± 0.44a
Ag NPs 150 mg/kg	14	41.17 ± 0.75b	1.40 ± 1.47b	3.73 ± 1.10a

These results showed that the rats that are given 150 mg/kg of silver nanoparticles for seven and fourteen days had significantly higher levels of urea in their blood ($P \leq 0.05$) than the rats that are in the control group (table 3). The readings represent the average value plus or minus the standard error. When letters are the same vertically, it means that there are no significant differences between the readings ($P > 0.05$). However, if the letters are different, it indicates that there are significant variations between the readings ($P \leq 0.05$). The results showed that the level of creatinine in the blood of rats that are given 150 mg/kg of silver nanoparticles for seven and fourteen days is significantly higher ($P \leq 0.05$) than that of the control group. The readings represent the average value plus or minus the standard error. When letters are the same vertically, it means that there are no significant differences between the readings ($P > 0.05$). However, if the letters are different, it indicates that there are significant variations between the readings ($P \leq 0.05$). At seven and fourteen days, there are no statistically significant differences in the serum uric acid concentration (mg/dL) between the groups of animals that are given 150 mg/kg of silver nanoparticles and those that are not (Table 3). The readings indicate the average value plus or minus the standard error. When the letters are the same vertically, it means that there are no significant discrepancies between the readings, with a p-value greater than 0.05.

Table 3 Average Diameters of Glomeruli, Renal Corpuscles, and Bowman's Capsule Space in Control Animals and Rats Injected with 150 mg/kg of Silver Nanoparticles for Seven and 14 Days.

Group	Injection Duration (Days)	Glomerular Diameter (μm) ± SE	Renal Corpuscle Diameter (μm) ± SE	Bowman's Capsule Space (μm) ± SE
Control	7	80.1 ± 0.8a	62.80 ± 4.90a	9.12 ± 0.56a
Ag NPs 150 mg/kg	7	53.96 ± 1.69a	68.50 ± 0.70a	14.54 ± 1.51b
Control	14	61.86 ± 3.09a	69.59 ± 49a	7.74 ± 0.99a
Ag NPs 150 mg/kg	14	49.91 ± 3.60b	65.01 ± 1.1.a	15.10 ± 1.18b

The readings are the mean ± standard error. Different letters indicate significant differences between readings ($P \leq 0.05$), while vertically similar letters show no significant differences ($P > 0.05$). This study found significant histological and biometric alterations in silver nanoparticle-injected mice' kidney tissues. Glomerular Diameter The group injected with 150 mg/kg silver nanoparticles for 14 days showed a significant decrease ($P \leq 0.05$) in glomerular diameters compared to the control group. However, glomerular diameters did not differ ($P > 0.05$) from the control group after seven days of injection. and Bowman's capsule space, the diameter of the renal corpuscle For seven and 14 days, the control and treatment groups had similar renal corpuscle sizes ($P > 0.05$). In contrast, Bowman's capsule space

Exp. Theo. NANOTECHNOLOGY 9 (2025) 15-26
significantly increased (P≤0.05) in groups injected with 150 mg/kg silver nanoparticles for 7 and 14 days compared to control groups.

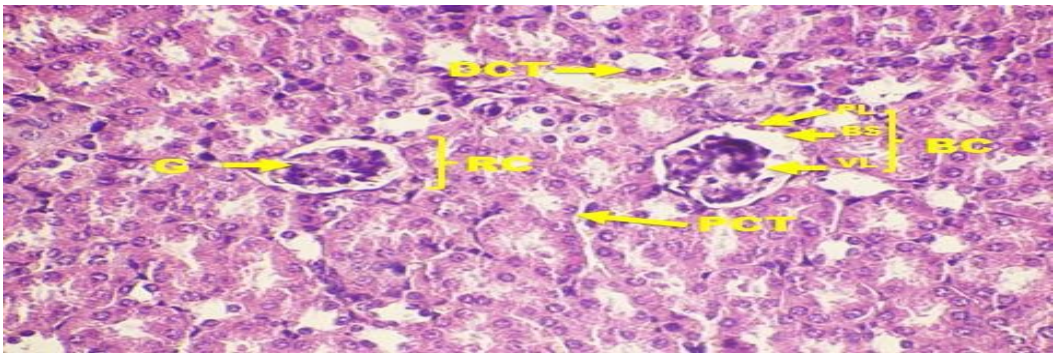

Figure 4 A cross-section in the kidneys of one of the mice from the control group shows the cortex part and contains the renal corpuscle (RC) renal glomerulus (G), proximal convoluted tubules (PCT), distal convoluted tubules (DCT), Bowman's capsule (BC), capillary vessels (PL), stellate renal capillaries (VL) and Bowman's space (BS) showing different arrangements and sizes at magnification X 400, H+E.

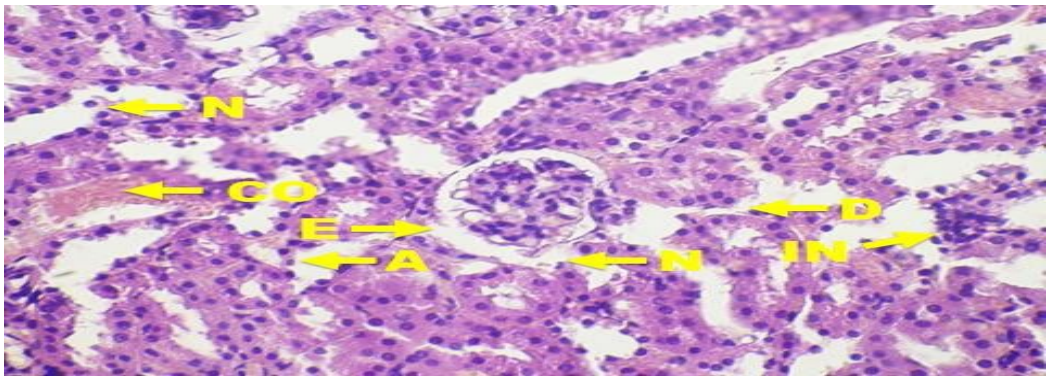

Figure 5 A cross section in the kidneys of one of the mice examined a group of samples treated with Ag nanoparticles, showing in the cortical region cell necrosis (N), vascular congestion (CO), cell infections (IN), damage to the tubule wall (D), disintegration of some nuclear cells (A), lysis of some (D), enlarged Bowman's vacuum (E) (X 400, H+E).

Exp. Theo. NANOTECHNOLOGY 9 (2025) 15-26

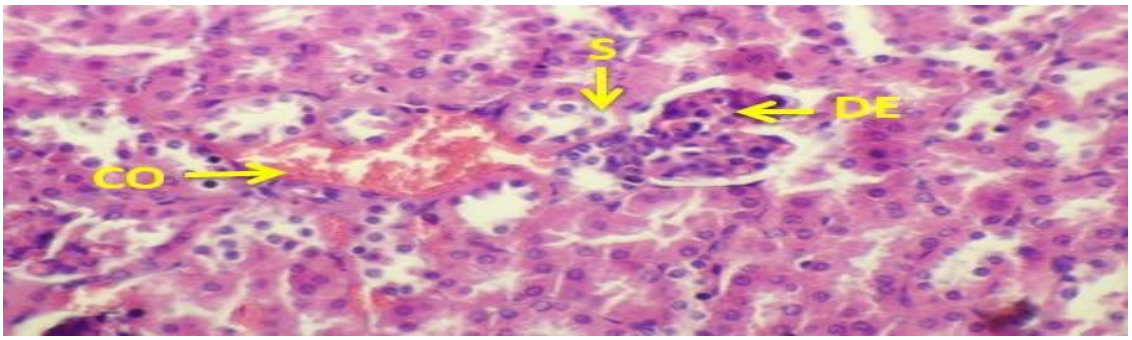

Figure 6 A cross-sectional section in the kidneys of one of the mice examined a group of samples treated with Ag nanoparticles, shows abnormal structure in the cortical region showing cell detachment from the basement membrane (s), vascular congestion (CO) and glomerular damage (DE) (X 400, H+E).

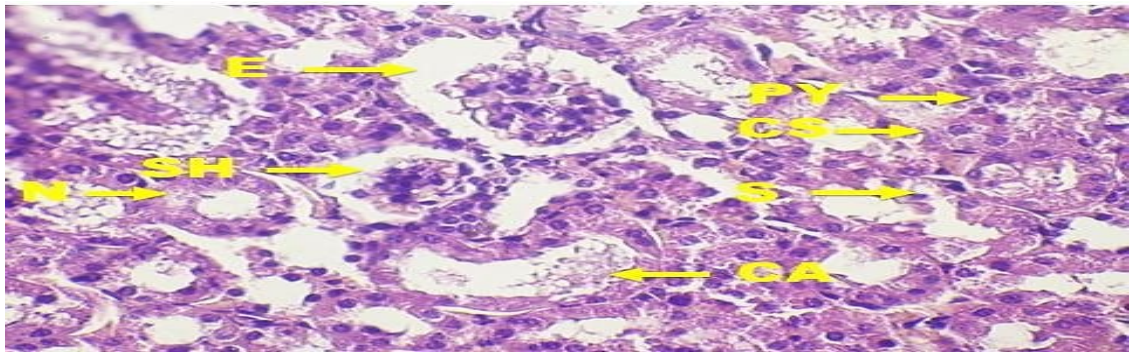

Figure 7 A cross-sectional section in the kidneys of one of the mice examined a group of samples treated with Ag nanoparticles, shows in the cortical region the expansion of the Bowman capsule (E), swelling and increased thickness of the cells lining the tubules (PY), contraction of some glomerular cells (SY), necrosis of their cells (N), calcium deposition in the tubules (Ca) (X 400, H+E).

The present study found that the diameters of the renal distal and proximal coiled tubules in the injected aggregates with 150 mg / kg of Ag nanoparticles for seven and 14 days are significantly higher than the control aggregates. Exposure to silver nanoparticles (AgNPs) caused histopathological changes in the kidneys of female albino rats, including glomerular degeneration. These findings suggest nanoparticles may be nephrotoxic. Silver nanoparticles (AgNPs) cause renal tissue oxidative stress, inflammation, and apoptosis, causing nephrotoxicity Apoptosis is triggered inflammatory cytokines are heightened kidney function is changed and oxidative stress is induced by AgNPs (21–24). These influences include the regulation of agents, expression of proteins linked to cell death, the investigation of renal tissue structure and markers demonstrating kidney function in the blood. From disrupting the architecture of tubular structures affecting by the loss of the brush border and damage to basal layers AgNPs likewise, lead to structural irregularities in the kidneys. The layout of the kidney along with alterations in brush borders and tubular basal layers can be influenced by silver nanoparticles (AgNPs) (2). Studies have indicated that silver nanoparticles elevate dialdehyde (MDA) levels in kidney tissues while reducing dismutase (SOD) activity (1). When pig kidney cells absorbed AgNPs coated with substances, reactive oxygen species and potential genotoxicity are observed necessitating biosafety estimations for products containing AgNPs [25]. Thoroughgoing understanding of how exposure to AgNPs influences both functionally on tissues is crucial for evaluating nanoparticle induced kidney toxicity and devising

preventive measures. The Understanding of the functional repercussions of nanoparticles on tissue is key, and the increase in swelling among cells within renal tubules may be attributable to this change, in diameter. Several research studies have shown that when given orally for 21 days at doses of 100, 250 and 500 mg/kg silver nanoparticles caused enlargement in tubules.

These findings underline the need to study nanoparticle effects on the kidneys and the mechanisms behind renal tissue changes. The brachial edge brush borders disappeared with this expansion due to epithelial cell loss in proximal renal tubules and renal corpuscle atrophy. Glomeruli may expand due to Bowman's capsule visceral layer breakdown or renal glomeruli contraction. Organ lining cell flattening. Calcium molds, epithelial cell nuclei hyperplasia, necrosis and death of some renal tubules, degeneration of epithelial cells lining the tubules, shrinkage, fragmentation, and loss of renal glomeruli are found in egg mouse kidneys [26-28]. After 7 and 14 days of additional Ag nanoparticle injections, these changes occurred. Reactive oxygen species and renal impairment may produce these changes. Small substances in the kidneys induce toxicity and malfunction. Capillary neutrophil clustering. Hematopoiesis expands and preserves blood vessels and creates cells in the glomerulus and renal tubules. ROS and oxidative acids are created by inflammation around blood vessels, renal corpuscles, and tubules. Increased proxy produces these compounds.

4. CONCLUSIONS

This study showed that the phenotypic, behavior, physiology, and histology of white Swiss mice may all be significantly altered by the presence of silver nanoparticles. The findings highlight the significance of carefully assessing any potential risks associated with silver nanoparticle exposure, particularly with regard to renal function and general health. the best results The Ag NPs group significantly lost weight after 14 days compared to the control group, as measured by weight measurements. The Ag NPs group also showed significant increases in urea and creatinine levels after 7 and 14 days, suggesting possible kidney damage. Histological analysis also revealed a decrease in glomerular diameter and an increase in Bowman's capsule space, further supporting the presence of nephrotoxicity. Mice injected with 150 mg/kg Ag NPs showed increased lethargy, introversion, back hunching, and appetite loss. To further understand the mechanisms underlying these effects and develop mitigation techniques for potential risks associated with the use of silver nanoparticles in various applications, more study is necessary.

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