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In Vivo Induced Nephrotoxicity of Silver Nanoparticles in Rat after Oral Administration

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ABSTRACT

Improvement in nanotechnology has identified promising silver nanoparticles (AgNPs) for many biomedicine applications. To assess the toxicity of silver nanoparticles in vivo, histopatological examinations of experimental mice were studied. Forty adult male Sprague-Dawley rats were randomly divided into five groups and orally treated AgNPs in different doses (30,125,300,700 mg/kg) during the 28-days. After paraffin embedding and hematoxylin and eosin (H&E) and periodic acid Schiff (PAS) staining, histopathological changes evaluated in kidney using light micrographs. The obtained results showed a decrease in diameter of convoluted tubules, glomerulus diameter, Bowman's space thickness and number of mesangeal cells in 30 and 125 and 300 mg/kg treated groups. These changes are more evident in 125 mg/kg of AgNPs group (P<0.05). The other histological changes in the tubules of rats affected by AgNPs included loose of brush border, basement membrane irregularity, necrosis, vacuolar degeneration and Congestion. The other glomerular and interstital alterations of rats affected by AgNPs included: basement membrane thickness, accumulation of mesangial matrix, necrosis and infiltration of inflammatory cells. These histological alterations were also more evident in rats exposed to 125 mg/kg of AgNPs. In 700 mg/kg group, no major changes in the structural component of the kidney were observed while occasional foci of inflammatory cell infiltrates were present.

Keywords: Kidney, Silver Nanoparticles, Histopathological Changes, Nephrotoxicity, Rat

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INTRODUCTION

Recent progress in nanotechnology deeply improved the potential applications in the domestic, industrial, and biomedical fields. Among several metallic nanoparticles that are involved in daily supplications, silver nanoparticles (AgNPs) is the most frequently used because of to their special chemical, physical and biological properties[1]. The number of AgNPs-containing products has grown from less than 30 in 2006 to over 435 in the 2015 [2].

There are growing danger about elevated industrial wastes for nanoparticles in soil and water and environmental exposure to these materials [3, 4]. Due to small sizes of AgNPs, the silver can entry into human body via different

routs including; ingestion[5], inhalation[6] and skin[7]. Depending on the size and concentration, accumulation of silver nanoparticles has been reported in various organ such as: kidneys, lungs, nervous system and liver [8, 9]. In vitro and in vivo evidences support the suggestion that AgNPs induce cytotoxicity and inflammatory effects in different kinds of cells and tissues [8, 10, 11].

Previously published study resulted that numerous metallic elements have toxic effect on kidney that preferentially accumulate and produce cellular injury in the this organ[12, 13]. Kim *et al*, demonstrated that, in rats after ingestion, silver nanoparticles are efficiently internalized in the glomeruli and basement membranes of renal tubules[9]. Chen *et al* showed histological damage to proximal tubular cells in mice exposed via oral administration to copper NPs[14]. Abdelhalim *et al*., also observed nephrotoxic effect of gold nanoparticles on the histochemical and histological alterations in the renal tissues [15]. Gui *et al* showed nephrotoxicity of titanium dioxide nanoparticles, assessed by physiological and gene expression modifications in mice exposed via oral gavage[16].

Production of reactive oxygen species (ROS) and the release of cytokines are suspected to be the mechanisms by which metal nanomaterials induce toxicity which in turn triggers cellular changes including DNA damage and apoptosis[17].

While nanotoxicity research is now gaining attention, but possible histological alterations in the renal tissues following exposure to AgNPs in animals and humans remain unclear. In this study, renal histopathological changes of rats were exposed for 28 consecutive days to AgNPs on 30, 125, 300 and 700 mg/kg body weight were studied.

MATERIALS AND METHODS

Animals

The protocol of this study was approved by the ethics committee of Hamadan University of Medical Sciences. Forty male Sprague-Dawley rats weighing between 180 - 200 g were obtained from Hamadan Medical University (Hamadan, Iran), and were housed in the animal house under controlled environment of 21±2°C and 50±15% humidity with a 12-h light/dark cycle. The animals were allowed free access to food and water during the 28-days of the experimental period.

Nanosilver Particles

AgNPs (CAS No. 7440-22-4) were purchased in powder form from US Research Nanomaterials, Inc. (Houston, TX, USA).). The purity of the nanosilver particles was more than 99%. Ag NPs powder was suspended as described in the previous study[18]. Deionized water was used for the dispersion of Ag-NPs into concentrations of 30, 125, 300, and 700 mg by vigorous vortexing, followed by sonication for 5 min. The particle-size distribution (PSD) of the Ag-NPs was analyzed using dynamic light-scattering instrument (DLS; Malvern, Nano ZS ZEN- 3600, UK).

Experimental design

The rats were randomly divided into five groups (n=8 in each). Animals in four groups were treated with 30, 125, 300, or 700 mg/kg AgNPs solution for 28 days by oral gavage and the control group received equal volumes of deionized water. The day after the last administration, body weight was measured and then rats were sacrificed, two kidneys of each rats were quickly harvested, excised and immersed in 10% neutral buffered formalin solution for histopathological analysis.

Histopathological Study

The fixed tissues were processed routinely, embedded in paraffin, sectioned, deparaffinized and rehydrated. Sections were cut into 5 um sections by the rotary microtome, mounted and stained with hematoxylin and eosin using the standard technique. Special staining techniques, periodic acid schiff (PAS) stained to assess the glomerular changes and tubular basal lamina and brush border. All the images were obtained through a transmitted light microscope (Nikon E800 research microscope) with video camera (motic 2000) and motic images 2.0 software for the acquisition of the differences in histomorphology of all studied groups.

Statistical analysis

Statistical analysis was performed using the SPSS 11.0 soft-ware, and the data are shown as mean± standard deviation (SD). One-way analysis of variance and Tukey test was used to determine differences between groups. To compare selected pairs of groups, p-value less than 0.05 was considered significant.

RESULTS

In this study no mortality occurred in any of the experimental groups, and no alterations were observed in the appearance and behavior of AgNPs treated rats in comparison with the control ones.

Characterization of silver nanoparticles

To identify the diameter of the silver nanoparticles, the AgNPs suspension was subjected to dynamic light scattering (DLS) analysis. It demonstrated a hydrodynamic diameter peak, with an average size of 200-300 nm, which was considerably larger than that indicated in product's information (< 100 nm).

Histopathological changes

Histopathological changes were evaluated in three components: Tubular, Glomerular and Interstitial. The sections from control rats showed that structures of renal tissue including, proximal and distal convoluted tubules, renal corpuscles and the interstitial tissue of tubules were intact and no cellular and tissue damages were found (Figs.1 and 2).

Tubular alterations

For the evaluation of the toxicity of AgNPs on renal tubules, basic parameters including: diameter of renal proximal and distal tubules were measured, other tubular changes as Brush border, basement membrane, necrosis, dissociation of cells, cloudy swelling, vacuolization, nuclear changes (anisokaryosis, pyknosis, karyorrhexis and karyolysis) and formation of Hyaline cast, were also qualified on basis of grading was used to score the histological alterations: (−) absent; (+) mild; (++) moderate; (+++) severe (Fig. 1, Table 1).

Diameter of renal proximal and distal tubules

As shown in Fig 3, the mean diameter of proximal tubule in the experimental groups of 30, 125 and 300 mg/kg significantly decreased compared to the controls ($p<0.05$) and the mean diameter of distal tubule in the groups of 125 and 300 mg/kg significantly decreased compared to the controls ($p<0.05$).

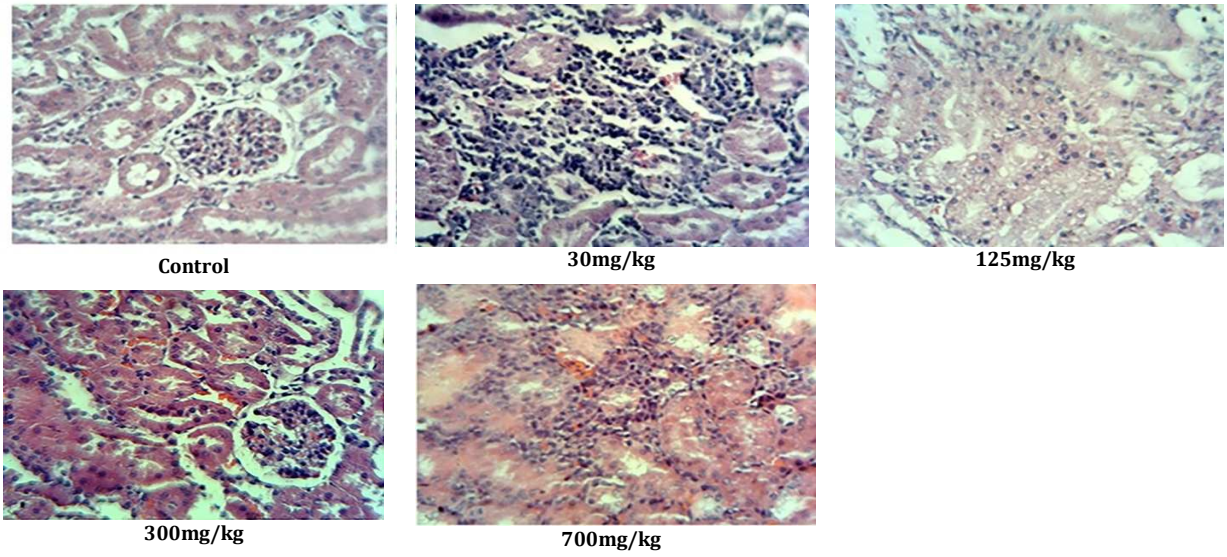


Figure 1:Light micrographs sections for tubular analysis of cortical region in kidney from control and 30, 125, 300, 700 mg/kg AgNS treated rats. Foamy degeneration in the vacuolated epithelial cells, congestion of the capillary loops and infiltration of inflammatory cells are shown. Haematoxylin& eosin (H&E) × 400

PAS staining

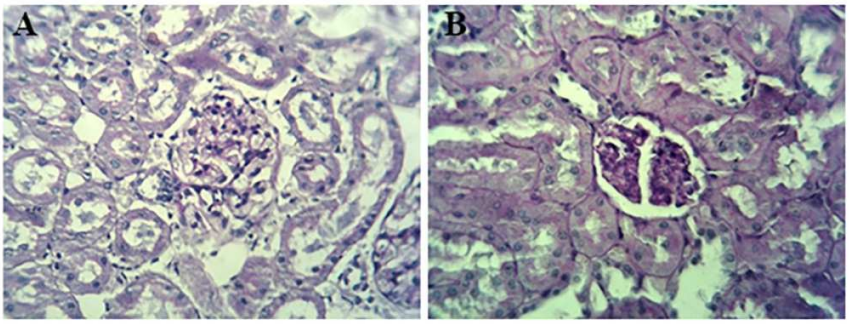


Figure 2: Light micrographs sections of cortical region in kidney from 30 mg/kg (A) and control (B) AgNS treated rats. Brush border looseness and basement membrane irregularity were seen in30 mg/kg AgNS treated rats. Periodic acid Schiff (PAS) × 400

Table 1: Effec of AgNPs (30,125,300,700 mg/kg) for 28 days on tubular qualified criteria; The following grading scheme was used to score the histological alterations: (−) absent; (+) mild; (++) moderate; (+++) severe

	Control	30 mg/kg	125 mg/kg	300 mg/kg	700 mg/kg
Brush border looseness	-	++	++	+	+
Basement membrane irregularity	-	+	++	+	+
Necrosis	-	++	+++	+	++
Dissociation of cells	-	++	+++	+	++
Cloudy swelling	-	+	++	+	+
Vacuolar degeneration	-	+	++	+	++
Anisokaryosis	-	+++	++	+	+
Nuclear pyknosis	-	+++	++	+	+
Karyolysis	-	++	++	-	+
Hyaline cast	-	+	+	-	-
Infiltration	-	+++	++	+	+
Congestion	-	+	+++	+	++

Proximal and distal diameter of tubules

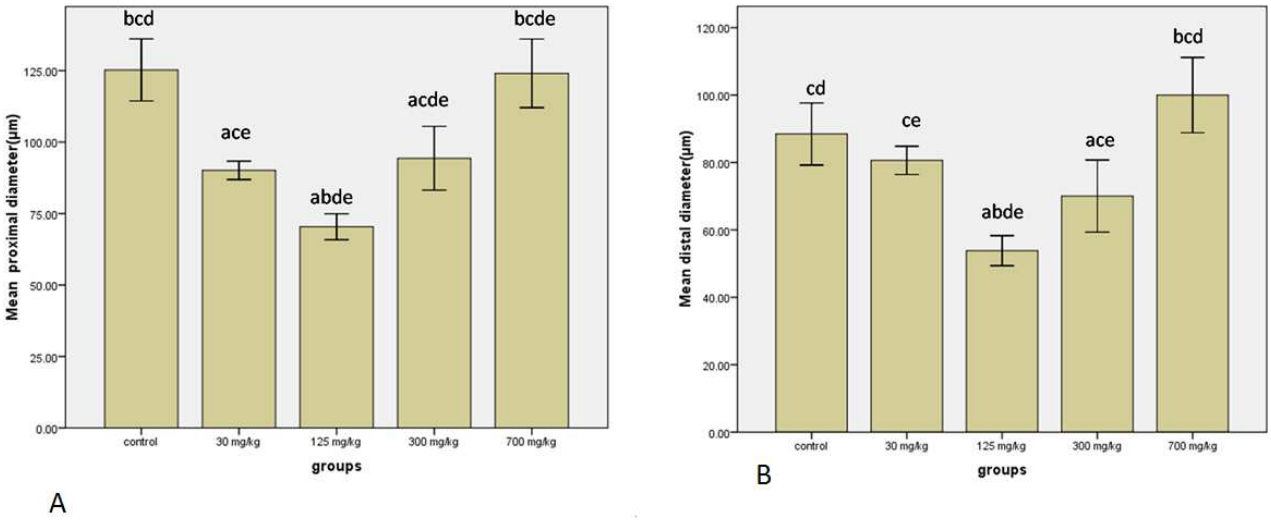
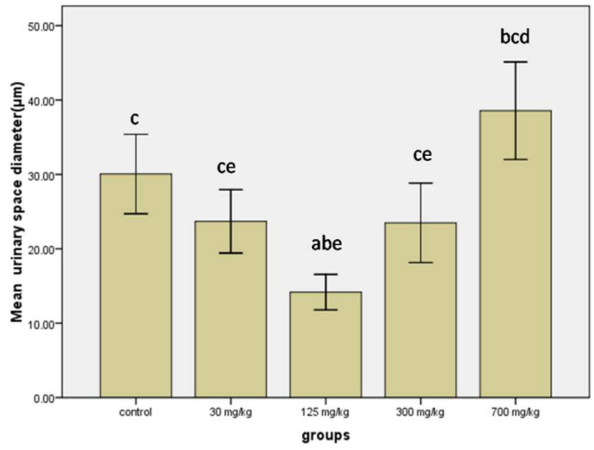
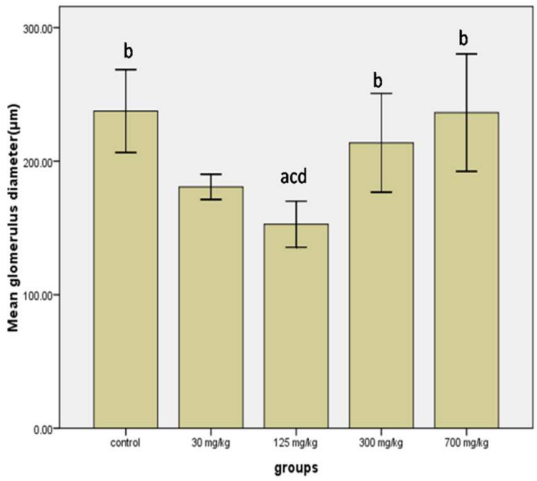


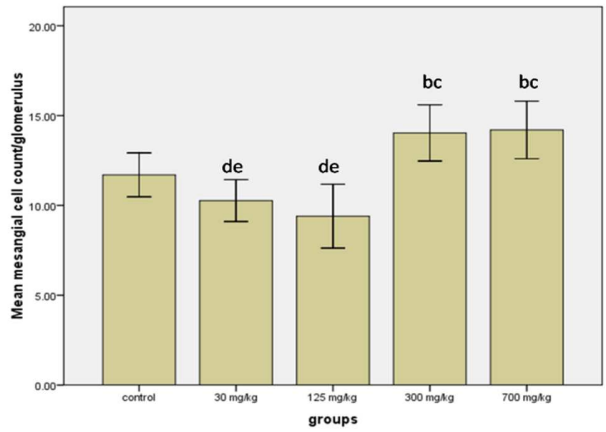
Figure 3: Effect of different doses of AgNPs (30,125,300,700 mg/kg) on diameter of tubules, proximal (A) and distal (B). All data are presented in Mean ± SD. There were significant differences (P<0.05) between treated groups and relation to control group that has shown with a,b,c.a: compared to control group (P=0.00), b: compared to 30mg/kg group (P=0.00), c: compared to 125mg/kg group (P=0.00). d:compared to 700mg/kg group (P=0.00), e: compared to 700 mg/kg group



A



B



C

Figure 4: Effect of different doses of AgNPs (30,125,300,700 mg/kg) on u space thickness (A), glomerulus diameter (B) and mesangeal cell number (C). All data are presented in Mean ± SD. There were significant differences (P<0.05) between treated groups and relation to control group that has shown with a,b,c,d. a: compared to control group, b: compared to 30 mg/kg group, c: compared to 125 mg/kg group, d: compared to 300mg/kg group, e: compared to 700mg/kg group

Table 2:Effect of AgNPs (30,125,300,700 mg/kg) on glomerular qualified criteria; The following grading scheme was used to score the histological alterations: (–) absent; (+) mild; (++) moderate; (+++) severe

GroupsAgNs(mg/kg) / Glomerular changes& Stains	Control	30 mg/kg	125 mg/kg	300 mg/kg	700 mg/kg
Accumulation of mesangial matrix	-	++	+	+	+
Hypercellularity	-	+	++	-	+
Necrosis	-	+	+	+	++
Congestion	-	+	++	+	++
Infiltration of inflammatory cells	-	-	-	-	-
Hyalinization	-	-	-	-	-

Glomerular alterations

For the evaluation of the toxicity of AgNPs on renal corpuscles, some parameters including: bowman's space thickness, glomerulus diameter, glomerular basement membrane thickness, number of mesangeal cell were measured and also glomerular changes as accumulation of mesangeal matrix, hypercellularity, necrosis, Congestion and

infiltration of inflammatory cells were qualified on basis of grading was used to score the histological alterations: (−) absent; (+) mild; (++) moderate; (+++) severe (Table 2).

Bowman's space thickness
The mean thickness of renal space in the experimental groups 30, 125 and 300 mg/kg decreased compared to the controls. However this change was significant and more prominent in 125 mg/kg treated group (Fig 4).

Mesangeal cell number
As shown in Fig 4, The mean number of glomerular mesangeal cells per glomerolus in the experimental groups of 30, 125 mg/kg significantly decreased compared to the controls ($p<0.05$) and the mean number of glomerular mesangeal cells per section in the groups of 300 and 700 mg/kg insignificantly decreased compared to the controls ($p>0.05$).

Glomerulus diameter
As shown in fig 4, the mean glomerular diameter in the experimental group of 125 mg/kg significantly decreased compared to the controls ($p<0.05$).

Interstitial tissue alterations
For the assessment of the toxicity of AgNPs on interstitial tissue, some parameters including: Congestion, infiltration of inflammatory cells was studied. These histological alterations were also more evident in rats exposed to 30 and 125 mg/kg of AgNPs.

DISCUSSION

Nanotechnology improvements' have identified potential candidates for many biomedical and biomedicine applications. AgNPs have entered the body through inhalation, ingestion, injection and dermal contact, resulting increase of silver concentration in various organs. However, an increasing number of reports suggest that AgNPs may be potentially toxic [19-21]. Some studies about the biodistribution of AgNPs in rats demonstrated a dose and size dependent accumulation of silver in different organ such as kidney [8, 19]. Therefore, AgNPs have the opportunity to interact with many stractural changes in this organ. Kim *et al* have reported that the AgNPs can dislocate into the rat kidneys following oral administration of AgNPs (60 nm particle size) over 28 and 90 days [8, 9]. In the present study, we extended the evidences to show orally AgNPs -treated (nominally < 100 nm diameter but actually ~200-300nm) adverse impact at kidney.

After intragasteric exposure to the AgNPs, histological structure of the rat kidney sections showed common pathological alterations in tubules, glomerulus and interstitial tissue. These alterations could conduct to the beginning of acute and chronic renal disorder.

Since glomerular filtration barrier, by having the primary site of blood filtration, is important in renal function, the basement membrane of was evaluated using PAS staining. Results showed that AgNPs could cause an increase in the thickness of basement membrane of glomerulus. In addition, congestion of blood cells in glomerulus and interstitial tissue was shown in morphological results of the present study. It is in agreement with the finding saying that blood flow directly depends on structural health and integrity of urinary filtration barrier[22, 23].

Results presented in this study confirmed that AgNPs administration caused marked changes in tubules in 125mg/kg group including degeneration and necrosis of the epithelial cells of tubules. These changes are accompanied by disorganization of proximal and distal tubules, loss of brush border and irregularity in the basal membrane, cloudy swelling, vacuolization, anisokaryosis, nuclear pyknosis and karyolysis epithelial lining cells and formation of hyaline casts. These alterations were more evident in the proximal convoluted tubules than the distal ones. The reason of these findings could be due to the fact that the proximal convoluted tubules are the primary sites of reabsorption and active transport. These alterations of the renal tissue of the AgNPs treated rats might show subacute renal injury. Kim *et al* have shown effects of AgNPs in rat on the function of kidney in the dose (125 mg/kg) during 90 days intragastric administration[8].

It has shown that tubular atrophy in diabetic nephropathy ultimately leads to end-stage renal disease[24]. Damage to the renal corpuscles indicated the widening of mesangial regions and accumulation of mesangial matrix, decreased the thickness of Bowman,s space and the diameter of

corpuscles. The Similar results also showed from work on toxic effect of gold nanoparticle[25].

Although the toxicity of nanoparticle is poorly understood, numerous in vitro and in vivo studies using different nanomaterials, have suggested that oxidative stress is a main reason of negative effect of nanoparticles use[26, 27]. The failure of homeostatic processes and generation of free radicals beyond the capacity of the body's defenses cause the oxidative stress occurs, resulting cellular injury and tissue damage[28]. Oxidative stress triggers the elevation of intracellular levels of reactive oxygen species (ROS). Conversely, the elevation in ROS in tissues is associated with destruction of vital molecules including DNA, lipids and proteins. ROS has a main role in the protection against exogenous chemical compounds. Therefore, elevated ROS levels can destruct cell and tissue structure and function and induce apoptosis or necrosis. These alterations can ultimately result in pathological changes and lead to organ dysfunction or cancers[29, 30]. ROS generation is a main mechanism of nanoparticles toxicity [31]. The unique properties of small size of of nanoparticles allows them to pass through cell membranes and other biological barriers and cause cellular dysfunction[18, 32]. The toxicological effects on kidney that have been showed in current study may be attributed to the same processes as suggested in studies mentioned above, although the particular mechanism needs further investigation.

In this study the histological alterations were more evident in rats exposed to 30 and 125 mg/kg of AgNPs. In 700 mg/kg group, no major changes in the structural part of the kidney were observed while occasional foci of inflammatory cell infiltrates were present. After oral administration, the small intestine is the first site for absorption of nanoparticles. The large agglomeration size of AgNPs in high concentration may also have prevented their absorption from small intestine and so resulted in an insufficient availability of AgNPs to the kidney and affect this organ. Similar changes were observed by Kim *et al* and Mahmoudian *et al* in liver [8, 11].

CONCLUSION

In conclusion, histological changes in of kidney by AgNPs exposure could be due to AgNPs toxicity. These results confirmed glomerular, tubular and interstitial degeneration was severing in 30 and 125mg/kg groups. One might conclude that these alterations are dose-dependent with lower ones induced more damage.

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