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Evaluation of the Toxicological Effect of Silver Nanoparticles in Male Albino Rats Kidney: Histopathological Study

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Abstract

Recently, silver nanoparticles (AgNPs) have been widely applied in different fields such as cosmetics, pharmaceutical and medicinal industries due to its easiness in absorption the gastro tract there for This study was designed and carried out to find the AgNPs effect on the structure and the performance of the kidney tissues on the male albino rats. To carry out the experiment, and achieve this study, twenty-four male albino rates were selected, and weight, and they were divided into 3 groups, the first onewas considered as a control group and injected with normal saline solution, while the second group(treatment) was injected withAgNPs 0.75 mg/kg of body weight, and the third group (treatment)was conjected with AgNPs 1.5mg/kg of body weight. Each concentration of AgNPs was dissolved in one ml of normal saline. All the injections were achieved as intraperitoneal injection for 30 days every day with 1 mL injection. The damage in the tissues of the kidney was appeared histologically in renal tubules as fatty degeneration, and vacuolation, in glomerulus appear pyknic nucleus, and also as massive aggregation around the blood vessels precisely in mononuclear cells with hemosiderin, and hemorrhage in renal tubules. Also appeared the amyloid in the blood vessels These symptoms can be recognized when a certain factorwas compared between the control group with infected with AgNPs, particularly at the level of the kidney function such as creatinine, uric acid and in the urea. The dysfunction and the changes in histopathology of the kidney is related to the toxic effect of the silver nanoparticles which cause the occurrence in ROS (Reactive Oxygen Species) which play an important role and cause disorder in kidney.

Key words: Histopathology, kidney, Silver Nanoparticles, Rat, Intraperitoneal.

Introduction

Nanotechnology use nanoparticles that has a grain size (average <100 nm) applied in different industries [1, 2]. The classification of the nanoparticles usually had done in accordance with its origin as categorized by European Commission mainly to two types; synthetic, and natural [3]. The most important property of the nanoparticles (NPs) are; high carrier capacity, small size, large surface area with relation to its small volume, these properties enable the NPs a very wide and open utilization [3, 4]. Recently, and due to the wide application of NPs such as biotechnology, drug developments, medicinal industries, therapeutics, and a lot more, a lot of questions raised in concerned with its effect on environment and health [5, 6]. Silver nanoparticles (AgNPs) has a certain and a very unique property such as; electric resistance, toxicity, as well as the surface Plasmon resonance [7]. Consequently, many researches were conducted to validate the multipurpose application such as antibacterial, anticancer, disinfectant agent, as well as in electronic devices [7, 8]. In recent time, the harmful and damaging effect on human organs like; neutrophils around portal area, monocytes aggregation, hepatocytes necrosis, it was also noticed that the nanoparticles may cause functional disorder in the liver enzymes, and reduce the testosterone levels in rats that treated with AgNPs[9, 10]. It was also found from recent studies that NPs can transfer and pass through gastro canal, lung and skin and causing infection [11, 12, 13]. Many studies also indicate that exposure and contacts with NPs is associated with many diseases like lung cancer, heart diseases, anaphylaxis leading to increasing in the mortality [14, 15], this is due to the migration of NPs via blood stream and allow them to reach distant sites, and precipitate and accumulated causing high toxicity, harms and damages to such sites like kidney damage that can be diagnosed through

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severity and predominance tests [16, 17, 18]The kidney damages were studied and reported by previous studied carried out on rats exposed and treated with AgNPs causing increases in the level of creatinine in the blood serum [19], also it was observed that the accumulation of AgNPs in females is much more than males (gender effect) [21, 22], which can be related to the regulation of the hormones [22]. The harmful effect is not only with AgNPs, but also there is a harmful effect on kidney with other toxic nanoparticles such as titanium nanoparticles, and iron nanoparticles [23, 24]. This encourage the researchers to carry out more studies about the toxic effect of nanoparticles of human health [25, 26]This study aimed to investigate the cytotoxic effect of AgNPs on the kidney(histology and function) with classify histopathological modifications as well as the disorder in the kidney functions.

Materials and Methods

Preparation of AgNPs solutions

The silver nanoparticles were brought from Sigma Alderich (with a grain size <100 nm), and then two concentrations were prepared 1.5 mg/kg and 0.75 mg/kg upon the body weight of each rat and according to [27] using physiology saline solution (conc. 0.09 Nacl) in dissolving the AgNPs

Investigated animals

Twenty-four Albino male rats weighting (weights 180- 200 g) at the age 8 weeks were selected and divided into 3 groups, each group contain 8 of them and were dosage and injected through intraperitonealwith 1 mL/day of AgNPs solution (every day 1 mL) for 30 days[28]and as the following;

- ➢ First group was dosage with only saline (control group)
- ➢ Second group was dosage with 0.75 mg/kg.
- ➢ . Third group was dosage with 1.5 mg/kg.

After 30 days, the blood was taken from each group of the albino male rats and was tested in the lab according to the protocol [27], were 5 ml of blood was obtained under sterile condition, then the serum of each sample was kept under -20 °C.

Biochemistry tests

1. **Creatinine concentration:** The estimation of creatinine was estimated using Agappe kits (from India) following the commercial protocol as depicted by the company itself.
2. **Urea concentration:** the estimation of urea was achieved according to the kits from the same Indian company (Agappe), and according to the protocol prescribed by the company
3. **Uric acid concentration:** the estimation of uric acid was determined following the same steps described in 1, and 2.

Histological preparation

After sacrificing the animals, the kidney was extracted from each Albino male rat, the kidney tissues were prepared for microscopic exam and taken pictures, the preparation steps were as the following [29]:

- Fixation,
- Dehydration,
- Clearing
- Embedding
- Sectioning
- Staining with hematoxylin and eosin.

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Statistics

The collected data was accomplishing with statistical analysis using ANOVA program to achieve the significant differences between groups using LSD test, and was considered at ($p<0.05$) [30].

Results and Discussion

Histopathology

No significant changes in the histopathology of the kidneys of control group (first group) as shown in figure [1], while the other groups can be explained as the following:For second group (dosage 0.75 mg/kg of AgNPs solution) shows vacuolar degeneration precisely in renal tubules, massive congestion in the blood vessels and architecture deformation in the tissue, fatty degeneration in glomerulus associated with pykinc as shown in figures [2 and 3]. This kidney damages is related to the toxicity of AgNPs that liberate aqueous silver ion in the organs that cause the changes in the kidney's cells due to its charge, elemental composition, shape, surface as well as the size which leads to oxidative environment inside the cells which affect the energy system and cause unbalance in redox process (due to the presence of free radical ions) that end with toxic effect, ROS and cause inflammatory that leads to cells fatal (death), these results matched with the findings of other researches [31, 32], The current research shows existence of fatty degeneration in the tubules cells of the urinary system, and this finding agreed with [33], who refer this to the disorder in the metabolism of the fatty acid due to the unbalance in the fatty acids amounts that enter and leave the living cells because of the cell member injury that caused by AgNPs that leads to vacuolation, and the pyknosis can be consider as necrosis step that appears in the renal glomeruli nuclei because of factors that caused death-inducing which stand for by the releasing the cytochrome C from mitochondria that indicates the rRNA losses from nuclei chromatin and the endoplasmic reticulum which cause the formation of the dark mass [34].For third group (dosage 1.5 mg/kg of AgNPs solution) shows the same fatty degeneration with necrosis in the renal tubules figure [4]. Also the kidney section shows the mononuclear cells with massive aggregation in the region of the blood vessels with hemorrhage and hemosiderin in the renal tubules. Also protein deposits appear in the tissue of the kidney section that appears in the blood vessels as amyloid figure [5].As depicted, pyknic with necrotic nucleus, architecture deformation in glomerulus figure [6]. All these damages, such as the necrosis caused by toxic effect of AgNPs as agreed by [35], which it was recorded that necrosis is caused by AgNPs due to the swelling of the organelles releasing lysate enzymes causing cell lysis inside the living cells in the presence of sodium and water, this swilling eventually will cause loss morphology of the mitochondria that induced oxidative phosphorylation disorder which end with ATP losses.In the current study, it was noticed deposition of amyloid in the blood vessels and this result agreed with [36], that refer this to the abnormal protein fibers is deposition in the blood vessels walls due to the defection in the transferring of the substances in and out of the living cell due to the AgNPs that liberate Ag^{+}(silver ion). The current study also find expansion in the blood vessels due to nerve stimulation effect which cause more blood to arrive with immune cells causing increases in the blood pressure inside the vessels due to the inflammation effect of the AgNPs that activate the chemical mediators [37]. The ruptures in the kidney's tissues is confirmed by the hemorrhage presences and this result agreed with the finding of [38] that indicates dosing albino rats with AgNPs will cause damages that appears as spreading and leakages in the red blood cells inside the space of interstitial.

Biochemistry

In the current study, the blood serum investigation has shown an important difference between the three groups in creatinine, uric acid, and urea as shown in table 1, due to the toxic effect of AgNPs, where creatinine and uric acid shows a significant increase in the groups injected with AgNPs, creatinine percentage in blood serum is considered as an important parameter to evaluate the kidney's function [39].Previous studies indicate that the accumulation, and deposition of AgNPs in medulla and cortical of the kidney, precisely in the mesangial cell of the cytoplasm [40, 41]. The changes in the morphology of the vascular component, as well as

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the modification in the cells of the renal mesangial pointed to the kidney's dysfunction like tubular dysfunction, damage in the glomerular filtration [42], and the main reason for this is the presence of radicals that liberated from AgNPs which cause cell oxidative stress causing ROS (Active Oxygen Species)

Table (1): Effect of silver nanoparticles on the creatinine, urea and uric acid levels

Groups (treatments)	**Mean ± SD**		
	Serum Creatinine mg/dl	Serum urea mg / dl	Serum uric acid mg / dl
Control	1.30 ±0.16	28.52±I.61	4.1±0.92
Low dose (0.75mg/kg- b.w)	3.61±0.26	39.42±2.16	8.62±1.82
High dose (1.5mg/kg - b.w)	4.68±0.86	51.45±5.21	10.23±1.83
LSD	0.28	2.86	1.86
Significant difference (p≤0.05) between groups			

Silver nanoparticles not only appears to be affected negatively on eukaryotic cells, but also in the prokaryotes, as a local study showed down regulation in gene expression of some virulence factors belong to Proteus caused direct effect of Ag nanoparticles [43].

Conclusion

Silver nanoparticles have dangerous effects on human body and environment, it absorbed by the digestive system and transferred via blood stream to different body parts and accumulated in the kidney.Transferring

Figure (1): cross section in kidney of control animals shows normal all architecture of tissue (H&E stain 40X)

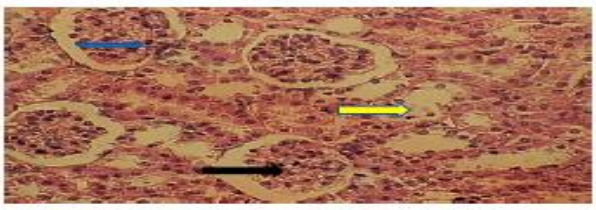

Figure (2) Cross Section in Kidney of Animal treated with AgNPs 0.75mg/kg. Shows vacuolar degeneration in renal tubules with pyknic and necrotic nucleus in glomerulus with fatty degeneration (H&E stain 40X)

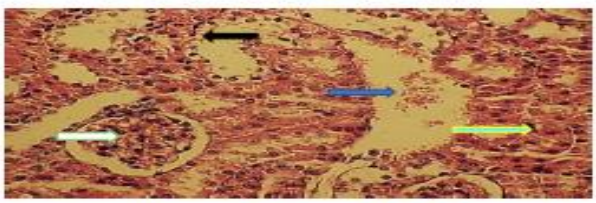

Figure (3) Cross Section in Kidney of Animal Treated with AgNPs 0.75mg/kg. Exhibited massive congestion of blood vessels with vacuolar degeneration of renal tubules and pyknic with necrotic nucleus in glomerulus with deformed architecture of tissue (H&E stain 40X).

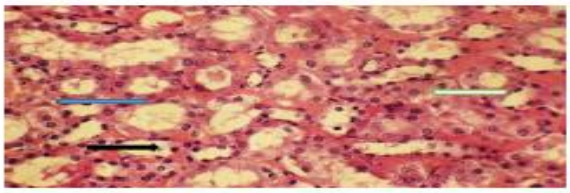

Figure (4) Cross Section in kidney of anima treated with AgNPs 1.5mg/kg. Exhibited fatty degeneration and necrosis of renal tubules and deformed architecture of tissues (H&E stain 40X)

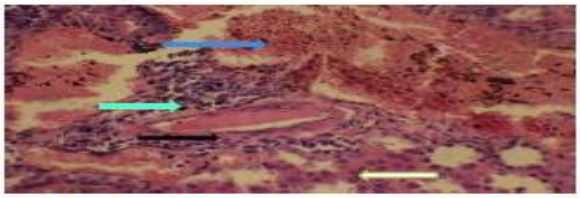

Figure (5) Cross in the kidney of animal treated with AgNPs 1.5mg/kg. Shows massive aggregation of mononuclear around blood vessels and hemosiderin with hemorrhage in renal tubules also shows Amyloid (H&E stain 40X).

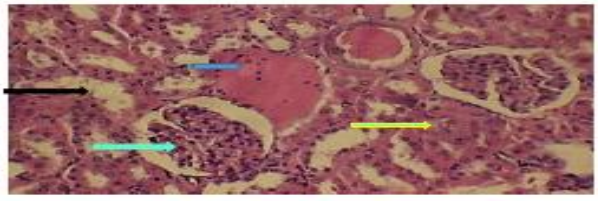

Figure (6) Cross Section in the kidney of animal treated with AgNPs 1.5mg/kg. Exhibited pyknic and necrotic nucleus with deformed architecture in glomerulus also dilated of renal tubules contain densely eosinophilic colloid casts and fatty degeneration with hemorrhage (H&E stain 40X).

and accumulation of AgNPs cause damages to blood vessels, tissues, in particular the kidney causing fatty degeneration and vacuolar in renal tubules as well as hemosiderin, hemorrhage, and protein deposes.Due to the presence of AgNPs in kidney's tissues, massive aggregation, and glomerulus in pyknic nucleus will appear.The increasing in the percentages of creatinine, uric acid, and urea in the groups treated with AgNPs indicates the kidney's disorder.

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