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Evaluation Of Histological Changes In Kidney Of Male Albino Rats Treated With Silver Nanoparticles

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ABSTRACT

The wide uses of silver nanoparticles in the industrial, medical and cosmetic fields with it ease of entry into human body and absorption by gastrointestinal tract there for designed this study to find out the effect of silver nanoparticles on the tissue structure and functional performance of the kidney, because the process of filtering blood takes place widely in the kidney. 24 male albino rats were used for the experimental study, the rats were divided into three groups, first groups(control) dosage with normal saline solution (Injected intraperitoneal), second group (treatment) dosage with AgNPs0.75mg/kg of body weight and third group (treatment) dosage with AgNPs 1.5mg/kg of body weight. Each concentration of AgNP was dissolved in1ml of normal saline and all experimental male rats were injected via intraperitoneal with single dose /day for30 days. The resultsin this study showed tissue damage in the kidney such as vacuolar and fatty degeneration in renal tubules with pyknic nucleus in glomerulus also appear massive aggregation of mononuclear cells around blood vessels with hemorrhage and hemosiderin in renal tubules with occurrence the amyloid inside the blood vessels with necrosis in different cells associated with malfunction of kidney Shown in significant difference in urea, uric acid and creatinine when compare with control group. Histopathological changes associated with the dysfunction of the kidneys due to the effect of nanoparticles directly on the cells such as the occurrence of the reactive oxygen species (ROS) generation has key role in the events of structural and functional disorders in the kidney of male albino rats.

Keywords: service quality, behavioral intention, emotional satisfaction, childcare center

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INTRODUCTION

Nanotechnology includes nanoparticles are represented by clusters of atoms whose average size less than100 nm [1,2]. Nanomaterials are classified according to their origin, including natural nanoparticles , synthetic nanomaterials and nanomaterials that are designed or engineered according of the European commission[3].Nanomaterials have unique properties such as their large surface area to volume ratio, small size, high carrier capacity, therefore it wide utilization [3,4]. To same reasons the unique properties of nanomaterials, which in turn led to the spread of their application, have raised many questions about health and environmental damages as a result of exposure to these materials during manufacture or use for consumption [5].Nanotechnology in last two decades has been seen penetrated in the different fields such as therapeutics, medicine, drug developmental, environment and biotechnology[6].Unique characteristics of silver nanoparticles [AgNPs]such as surface plasmon resonance Toxicity and electric resistance [7]. Accordingly, many studies have been conducted to verify its properties and application for multiple purposes such as water disinfectants, anti-cancer agents' anti-bacterial agents are found in wound dressing and electronic devices [7,8].However, in spite of this, silver nanoparticles have harmful effects on the multiples organs such as causes necrosisof hepatocytes and aggregation of monocytes and neutrophils around portal areaalso causes functional disorders such as significant increase in the level of liver enzymes of male albino rats were treated with AgNPs[9]. Silver nanoparticles causes decreased of testosterone level when examining the blood serum of male albino rats [10]. Recent studies have been shown that small dimensions of material nanoparticles facilitate it to pass and translocation through natural barriers such as skin Lung and gastro-intestine canal and causes chronic and acute toxic infected [11,12,13]. Several epidemiological studies have shown that exposure to nanoparticles was associated with various diseases such as anaphylaxis, heart disease and lung cancer with an expected increase in mortality due to this nanoparticle materials [14,15]. Migration of nanoparticles by blood stream reaches to distant sites and accumulates there and causes toxicity with damage to these sites,such as plasma ultrafiltration that takes place in the renal glomeruli, may be exposed to these nanoparticles with epithelial cells of the renal tubules causes damage occurs to them [16]. Chronic kidney infected and acute kidney damage are major health complications which is characterized by its predominance and severity [17,18]. Reported when mister rats were exposed to treatment with silver nanoparticles damage was observed in the renal tubules and mitochondria with a significant increase in serum creatinine level was observed [19]. Accumulation of nanoscale silver particles in the kidney, due to gender-dependent difference several causes have been reported in which a higher concentration appeared in females compared to males [20,21]. That difference in the accumulation of the amount of AgNPs between the two sexes as mentioned in one of explanations is possible due to hormonal regulation in the kidneys of experimental rats [22]. There more harmful effects that accrue as result the interactions of chemical with toxic of silver nanoparticles with other metallic nanoparticles such as Fe, TiO and Ag [23,24]. Humans exposure to the toxic effects of nanomaterials was recently acquired much interest in health field [25, 26].The aim of the present study is to investigate the toxicity effects of silver nanoparticles on the histological structure and functional ability of kidneys to identify histopathological changes and functional disorder upon exposure to AgNPs.

Material and Method

Preparation of silver nanoparticles

Nano powder of silver nanoparticles (AgNPs)<100nm particles size was purchased from Sigma- Aldrich chemical company[USA].In order to prepare silver nanoparticles for use according to the requirements of the experiment have been prepared, it was divided into 0.75mg/km and 1.5mg/km of the body weight of each animals used in the experiment[27].each weight of AgNPs dissolved in 1ml of normal physiology solution(0.09NaCl)

Experimental animals

24 male albino rats at the age of 8 weeks, weighting (200-210) grams were used. Each 4 rats were placed in cage, the animals have been randomly divided into three main groups each group consisted of 8rats. The first group(control) was dosagewith (1) mlnormal saline solution injected via intraperitoneal, second group was dosage with AgNPs0.75mg/kg of body weight, third group was dosage with AgNPs 1.5mg/kg of body weight for 30 days by single dose/day, all experimental animals given the AgNPs via injected intraperitoneally [28].At end the experiment all male albino rats have been sacrificed according to the protocol following in dealing with laboratory animals were anesthetized with either. Blood was obtained in an amount of 5ml from the left ventricles of the heart of each rat under sterile conditions, the blood samples were placed in laboratory tubes devoid of heparin ,in which the serum was preserved at $20C^0$- after a centrifugation process at 5000rpm to determine the effectiveness of the kidneys function [27].

Samples preparations

Biochemical preparation

Include the estimation of kidney functions test of the following

A- Estimation of creatinine concentration;

The level of creatinine concentration in the serum of the experimental animals was determined by using the procedure with a commercially kit from Agappe company-India.

B- Estimation of urea concentration;

To determination the level of the urea concentration in the serum of the experimental animals was used the procedure with a commercial kit from

Agappe company-India

C- Estimation of uric acid concentration;

The level of uric acid concentration in the serum of the experimental animals was determined by using the procedure with a commercially kit from Agappe company-India.

Histological preparation

The kidney was extracted from each experimental animal, the tissue slides were prepared in several stages, including fixation, dehydration, clearing, embedding, sectioning. staining with eosin and hematoxylin and using the microscope for examination and photography [29].

Statistical analysis

The comparative study that was conducted on the experimental groups show results have been subjected to statistical analysis between the groups taking into account the effectiveness of the kidneys functions, analysis of variance (ANOVA)was used in the statistical analysis of all values have been obtained with least significant difference (LSD)Test. The data was considered as significant difference with expressed as the mean ± SD<0.05.[30]

RESULTS AND DISCUSSION

Histopathological study

The results of the study showed no histopathological changes in the kidney of control group figure [1], while histopathological changes occurred in experimental groups that were dosed with silver nanoparticles and in varying proportions as following;

-The sections of kidney in the animals group was treated with AgNPs (0.75mg/kg-.b.w) appeared vacuolar degeneration in renal tubules and fatty degeneration with pyknic nucleus in glomerulus figure[2].In addition notes massive congestion of blood vessels with deformed architecture of tissue figure[3].

Silver nanoparticles cause side toxic effects on human and environment due to the

Ionic form of silver in aqueous phase entering the body organs leads to changes within the cells that cause inflammatory response this is consistent with [31],were clarified sliver nanoparticles have chemical (solubility, surface coating, elemental composition) and physical (surface charge, shape, size)properties that aid in the presence of chemical conditions for induction an oxidative environment within the cells. These conditions lead to an unbalanced in the cellular energy system which depends mainly on the ability of redox, which leads to initiate the inflammatory response or the possibility cell death. The entry of silvernanoparticles into the cells leads to state of oxidative stress in the cells, which due to the toxicity of nanoparticlesand liberates free radicals in the body to attack the cells membranes releasing the active oxygen species (ROS) that leads to occurrence of the inflammatory response [32]. The study showed the presence of fatty degeneration in the urinary tubules cellsagree with[33] where indicated the main cause of fatty degeneration is the occurrence of disturbances in fatty acid metabolism and an imbalance between the amount of fat entering and leaving cells due to the damage of the cell membrane caused by the silver nanoparticles, and thus the accumulation of fats inside the cells and the occurrence of vacuolation. The pyknosis is a stage of necrosis which appeared in the nuclei of the renal glomeruli due to the effect of death- inducing factors represented by cytochrome C released from mitochondria is due to the loss of rRNA from the endoplasmic reticulum and the chromatin of nuclei becomes condenser in the dark mass form, which represent one of the types of necrosis[34].

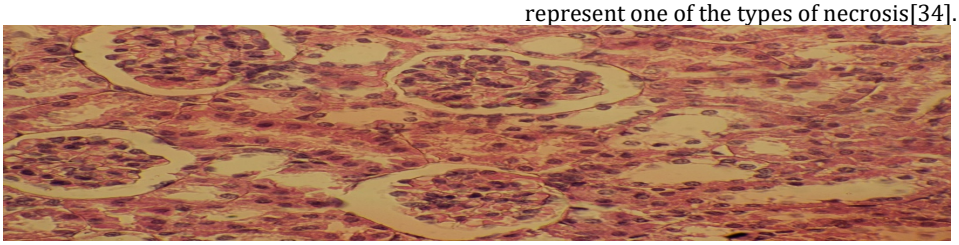

Figure (1): cross section in kidney of control animals shows normal all architecture of tissue (H&E stain 40X)

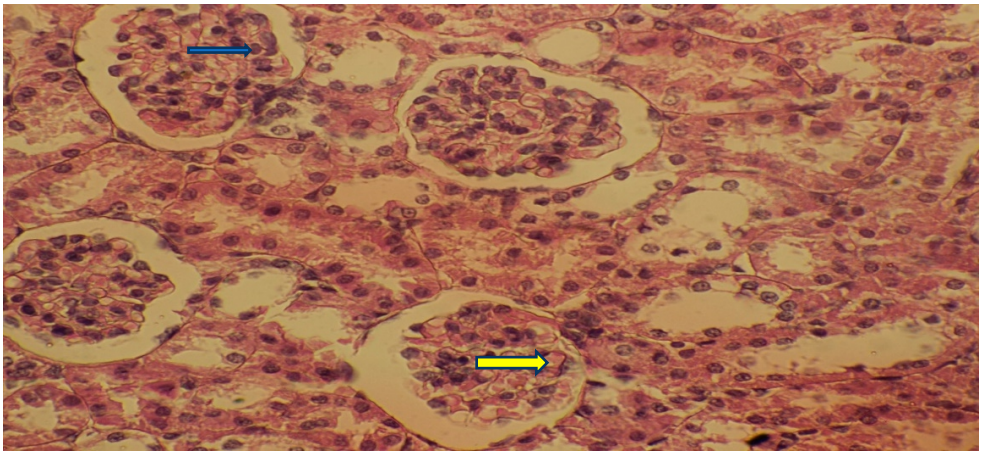

Figure (2) Cross Section in Kidney of Animal treated with AgNPs 0.75mg/kg. shows vacuolar degeneration in Renal tubules with pyknic and necrotic nucleus in glomerulus with fatty degeneration (H&E stain 40X)

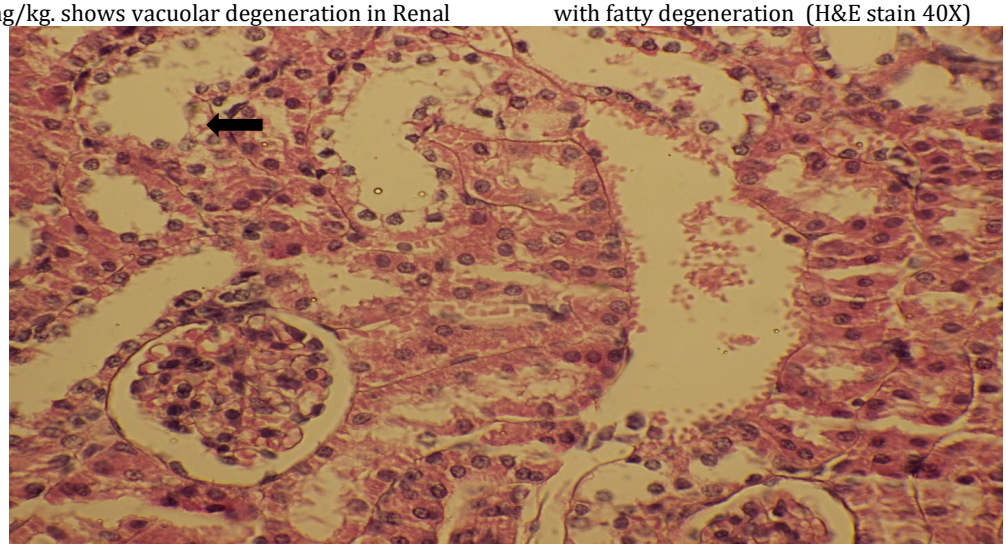

Figure (3) Cross Section in Kidney of Animal Treated with AgNPs 0.75mg/kg. Exhibited massive congestion of blood vessels with vacuolar degeneration of Renal tubules and pyknic with necrotic nucleus in glomeruluswith deformed architecture of tissue (H&E stain 40X)

-The kidney sections of animals group was treated with AgNPs(1.5mg/kg-b.w), the sections of the kidney exhibited fatty degeneration in renal tubules with necrosis figure [4]. Also showed massive aggregation of mononuclear cells around blood vessels with hemosiderin and hemorrhage in renal tubules, as well as the emergence of deposits of proteins represented by amyloid inside the blood vessels figure [5]. In addition to occurring the pyknic and necrotic nucleus with deformed architecture in glomerulus as well as the emergence of densely eosinophilic colloid casts in dilated of renal tubules with fatty degeneration figure [6]. Occurrence the necrosis in current study because the harmful effect of AgNPs in renal tubules in agreement with[35] where recorder the silver nanoparticles cause necrosis due to the entry of large quantities of water and sodium into the cells, that leads to swelling all organelles and continuation of the damage such as loss morphology of mitochondria with separation pf ribosomes from the rough endoplasmic reticulum, that leads to disorder of oxidative phosphorylation due to loss of ATP. Disturbance of the balance inside cells causes the breakdown of the all organelles and releases lysate enzymes that cause cell lysis. The resultsin this study showedamyloid deposition in the blood vessels and this is consistent with [36] as mentionedamyloid an abnormal fibrous proteins that is deposition in the walls of blood due to the defect of substances transfer between inside and outside of cells as a result of a defect in the construction of proteins by silver ions effect that liberated from the silver nanoparticles. The result also showed an expansion of the blood vessels as a result of treatment with AgNPs, the occurrence of an inflammatory response and the occurrence of a large blood flow in these blood vessels. The expansion of these vessels occurs due to the effect of nerve stimulation as a result of the presence of the inflammatory condition, which leads to the arrival of more blood carrying immune cells creates great pressure on the walls of the blood vessels and leads to their expansion and also lead to the activation of chemical mediators[37]. The presence of hemorrhage in the tissue sections of the kidney confirms the presence rupture in the tissues as a result of exposure to silver ions liberated from silver nanoparticles, this result consistent with[38] which indicated the doses of AgNPs leads to leakage and spread of red blood cells within the interstitial space in the damage tissues

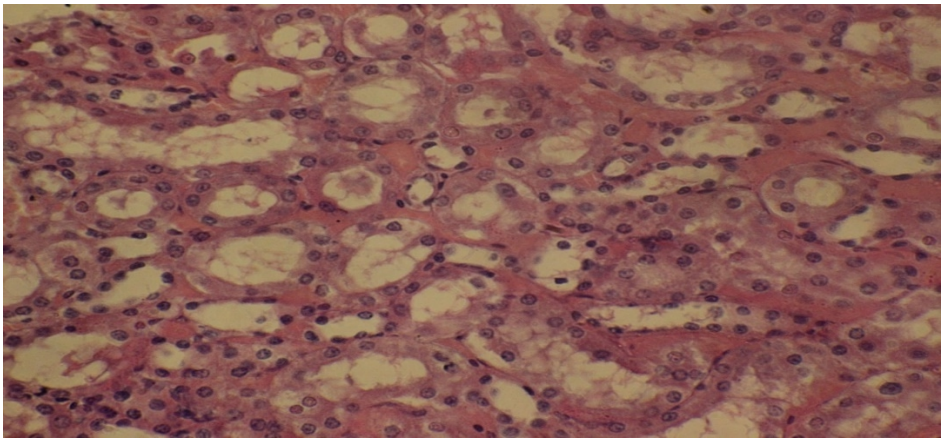

Figure (4) Cross Section in kidney of anima treated with AgNPs1.5mg/kg. exhibited fatty degeneration and necrosis of renal tubules and deformed architecture of tissues (H&E stain 40X)

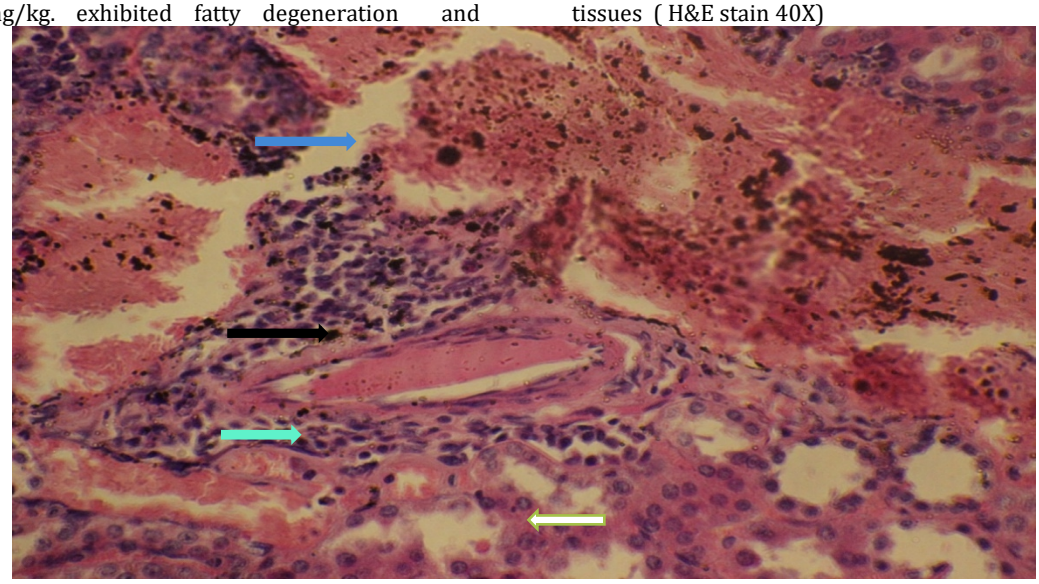

Figure (5) Cross in the kidney of animal treated with AgNPs 1.5mg/kg. Shows massive aggregation of mononuclear around blood vessels and hemosiderin with hemorrhage in renal tubules also shows Amyloid (H&E stain 40X)

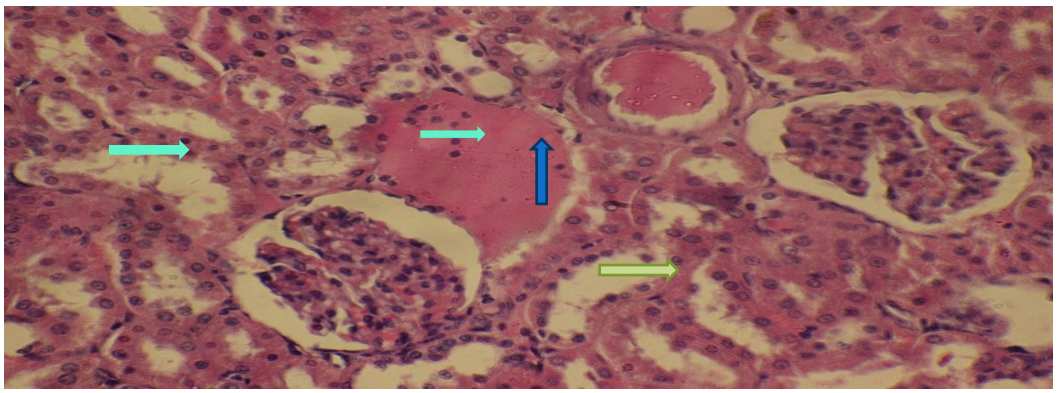

Figure (6) Cross Section in the kidney of animal treated with AgNPs 1.5mg/kg. exhibited pyknic and necrotic nucleus with deformed architecture in glomerulus dilated of renal tubules contain densely eosinophilic colloid casts and fatty degeneration with hemorrhage (H&E stain 40X)

Biochemical study

The investigation of blood serum in this study showed significant differences in urea, uric acid and creatinine when compare with control group as intable [1] due to nephrotoxicity induced via the toxic effect of AgNPs is companions with the increase in uric acid, urea and creatinine blood serum. Creatinine is important biochemical parameter in diagnosis the function disorders in kidneys [39]. The previous study pointed to accumulation of silver nanoparticles in the cortical and medulla of kidneys and deposited in the cytoplasm of mesangial cells of glomeruli [40-41]. The morphological changes of vascular component and modification of ultrastructural renal mesangial cells is parallel to the dysfunction of the kidneys such as glomerular filtration damage and tubular dysfunction [42]. Entry of silver nanoparticles into the cells leads to a state of oxidative stress in the cells due to the toxicity of nanoparticles and liberates free radicals in the body and causes the active

oxygen species (ROS) [31].Not only the eukaryotic cells appears to be affected negatively, but also in the prokaryotes, among many studies, a local report was showed down regulation in gene expression of some virulence factors belong to Proteus spp caused direct effect of Ag nanoparticles [43].

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

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			

CONCLUSIONS

Silver nanoparticles are a dangerous factor for the environment and humans as well, and their Danger lies in the ease of entry into the human body, absorption by the digestive system and Their transfer to various parts of the body, including the kidney where there is a process of Filtration of blood that is loaded with nanoparticles causing tissue damage associated with it Malfunction of the kidneytissue damage includes vacuolar and fatty degeneration in renal tubules With pyknic nucleus in glomerulus and also appear massive aggregation of mononuclear cellsAround blood vessels with hemorrhage and hemosiderin in renal tubules as well as the emergence Of deposes of proteins represented by amyloid inside the blood vessels with necrosis in different Cells. The malfunction of kidney shown in significant differences in urea, uric acid and creatinine When compare with control group, creatinine is important biochemical parameter in diagnosis the Function disorders in kidney.

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