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Research Article

Histological Comparative of Kidney of Neonatal Mice Exposed to Silver Nanoparticles During Fetal Development

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ABSTRACT

This study aimed to compare the histological changes in the neonatal kidney after their mothers exposed to different doses of silver nanoparticles colloidal solution (AgNPs) during the three stages of pregnancy. Pregnant Swiss albino mice (n=60) were randomly divided into three treated groups. They were intraperitoneally injected with AgNPs for 7 days during each stage of the gestational period. The newborn mice were sacrificed immediately after the birth, and the kidneys were being collected for histopathological analysis. The results showed that the AgNPs caused histological changes in the neonatal kidneys; vacuolation of some renal vesicles and cortical tubules, cystic tubular dilation, glomerular tuft shrinkage, and focal tubular necrosis in the first week-dose exposed pregnant. Disintegrating of immature glomeruli, distention of Bowman's space of mature glomeruli, tubular necrosis, loss of renal parenchyma, medullar tubules containing hyaline casts, and subcapsular haemorrhage in the second week-dose exposed pregnant. Massive hypercellularity in the deeper part of the renal cortex, cortical and medullary tubules dilation, atrophy of subcapsular immature tubules, cortical cyst formation, glomerular tuft necrosis, dilation of Bowman's space with evidence of crescent formation, and medullar portion replaced by scant loose connective tissue containing few numbers of tubules the third week-dose exposed pregnant. The results showed that the AgNPs has more negative effects on the kidney development at the third week-high dose and comparing the histological changes in the neonatal kidney were appeared in a time-depended manner and in a dose-depended manner. More researches must be carried out to obtain better understanding of AgNPs toxicity on fetal development and its ability as a teratogenic agent to induce external and internal abnormalities in the fetus.

Keywords: silver nanoparticles, neonatal mice, kidney, comparative histology, development.

INTRODUCTION

Nanosilver is an inorganic and manufactured nanoparticles that defined as tiny substances with one dimension at least and less than 100 nanometers[1]. It is more effective at nanometer size due to high ratio of surface area to volume. This lead to create a particle surface with excessive atoms which make them have a unique physiochemical properties utilized in numerous applications[2,3] especially in the products play an important role in modern medicine; such as an antimicrobial agent, sensors and fluorescent labels, therapeutic agents for treatment of several diseases, targeting of cells and transfection vectors, silver-coated catheters, and implantable medical devices. Moreover, silver nanoparticles have been used in the jewels, coin money, photography, dental alloys, etc., widely since ancient time[4-7]. AgNPs distributed throughout the human body tissues including placenta after being received to the blood stream via different ways; ingestion, inhalation, and dermal exposure[8]. Furthermore, the deposition and accumulation of the agglomerates of AgNPs in tissues depended on their diameter[9], and the degree of the AgNPs agglomeration along with their particle size, shape and surface modification can induce embryonic toxicity and abnormalities during embryogenesis[10]. On the other hand, AgNPs was identified in the cells of visceral yolk sac (extra-embryonic tissues), but accumulated slightly in the embryo when pregnant mice euthanized at 10 GD (gestational day) after the last intravenously injection (7-9 GD) with AgNPs (50 nm)[11]. NPs can reach the embryo across the placenta when pregnant rats exposed to it, causing developmental abnormalities especially in the critical times of organogenesis phase and the placenta formation during 10-13 (GD)[12]. Furthermore, the accumulation of nanosilver level in the organs of rat offspring which collected at 4 PND (postnatal day) after their mothers exposed to AgNPs (7.9 ± 0.95 nm) orally during gestation period was higher in the kidney than in brain, lung and liver the pregnant rats[13]. The present study aimed to comparing the histological changes in the neonatal kidney after their mothers exposed to different doses of silver nanoparticles colloidal solution (AgNPs) during the three stages of gestation period; implantation stage in the first week (1st week), organogenesis stage in the second week (2nd week), and fetal development with growth in the third week (3rd week).

MATERIALS AND METHODS

Silver preparation

Silver nanoparticles colloidal solution (4000 ppm) with size range (35-100) nm was purchased from Nano Pars

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Panda Company (Iran). Its size was measured by using Scanning Electron Microscope SEM. The stock solution was activiated by ultrasonicator immediately before the three doses prepared. The concentration (1, 5, and 10 ppm) was prepared by adding normal saline to a specific volume of stock solution[14,15]. The diluted solutions was activated by magnetic hotplate stirrer at 250 rpm for 15 min immediately before injecting the pregnant mice[16,17].

Animals Collection

Swiss albino mice (♀, ♂) were chosen from two breeding colonies Biotechnology Research Center/Al-Nahrain University and National Center for Drug Control and Research (NCDCR) in Baghdad. These mice were housed in plastic cages in a controlled room under optimum conditions of temperature (20-23) °C, enough relative humidity, and artificial light-dark cycle (7 am to 7 pm)[15,18]. After animals acclimatized to these conditions for 7 days with an easy access to a specific diet and drinking water. The first day of gestation (0 GD) was determined in the morning when 1 female with age (45-60) days and weight about 25-30 g housed with 1mature male overnight in a plastic cage by the presence of a vaginal plug[17,1,13].

Experimental design

The healthy pregnant mice (n=60) were randomly divided into three treated groups according to gestational stages to study the effects of silver nanoparticle colloidal solution on the kidney of newborn mice (age = 1 day) after their mothers expose to AgNPs for 7 days during each stage of the gestational period. Each stage of gestation includes 20 mice which in turn subdivided to three treated groups according to the dose of the AgNPs (1, 5 and 10 ppm) plus the control group. The pregnant mice were injected with AgNPs intraperitoneally (IP) once a day, every morning and the controled group were injected IP with normal saline (NS).

Histopathological analysis

After parturition, the neonatal kidneys were collected (one newborn mice from each litter) and fixed in 10% formalin immediately after the sacrifice by euthanasia[13,18,19]. After the steps of dehydrated with increasing series of gradual concentrations of ethanol and cleared with xylene finshed, the tissue embedded in molten paraffin wax and left to harden. The sections (5 µm thickness) were stained using H& E Stain according to[20]. The photos of the changes in the neonatal kidney tissues were taken via German Olympus Microscope / with a Japanese Digital Camera, canon type.

RESULTS

The principle renal findings of treated groups with three doses (1, 5, and 10 ppm) during 1st week of pregnancy comparing with control groups (fig. 1A) characterized by vacuolar degeneration of tubular epithelium mainly in the deeper part of the cortical zone. Marked cystic tubular dilation with severe degeneration of some renal vesicles in subcapsular nephrogenic zone observed in 1st week-low dose (fig. 1B), glomerular tuft shrinkage and focal tubular necrosis appeared as acidophilic structureless in 1st week-middle dose (fig. 1C), and focal tubular necrosis without evidence of glomerular alteration detected in1st week-high dose (fig. 1D).

The histological changes of neonatal kidney during 2sd week-low dose of pregnancy repersened by disintegrating of immature glomeruli while the mature glomeruli showed tuft vascular congestion and vacuolar degeneration of tubular epithelium with evidence of loose connective tissue (fig. 2A), in addition to the appearance of some renal tubules at the medullar zone containing eosinophilic hyaline casts (fig. 2B). 2sd week-middle dose showed severe degenerative changes and tubular necrosis result in marked loss of renal parenchyma with evidence of atrophy of surviving remnant tubules and slight distention of Bowman's space (fig. 2C) associated with the severe destruction of adjacent dilated tubles. 2sd week-high dose showed clear subcapsular haemorrhage with disruption of cortical tubules and some of the glomeruli which express little changes accompanied either with tubular dilation (fig. 2D) or tubular vacuolar degeneration.

The majority of renal glomerulus in 3rd week-low dose showed massive hypercellularity, mainly in the deeper part of renal cortex, as well as moderate tubular dilation were reported in both renal cortex and medulla (fig. 3A). The renal lesion of 3rd week-middle dose was more than the previous group which involve both renal tubules and glomeruli accompanied with severe atrophy of subcapsular immature tubules and cortical cyst formation (fig. 3B).The characteristic renal lesions of 3rd week-high dose showed severe necrosis in the glomerular tuft and evidence of vacuolation (fig. 3C), while other glomerular structures exhibit marked dilation of Bowman's space with evidence of crescent formation together with the severe loss of surrounding parenchyma, moreover, the medullar portion replaced by scant loose connective tissue containing few numbers of tubules (fig. 3D).

DISCUSSION

Recently, AgNPs used widely in many applications, and its ability to pass across the placenta was documented in several studies[21,18]. On the other hand, the effects of silver nanoparticles on renal development have gained the least attention. Accordingly, it is necessary to understand the silver toxicity on the kidneys of newborn mice and determines the developmental time points in which the exposure to AgNPs could be more harmful. To our knowledge, this study is the first that focused on comparing the histological changes in the kidney of newborn mice when exposed to different doses of silver nanoparticles during different developmental time pointsIn the present study, histological comparative of the neonatal kidney which their mothers exposed to different doses of AgNPs during the 1st week of gestation showed little changes compared with the other treatment and control groups in a time-depended manner. This alteration may be associated partly with the time of visceral yolk sac formation at GD 6 and before the placenta was formed at approximately GD (10-12) which allows the silver NPs transfer to embryo[22,11] and partly due to the nephrogenesis which begins at E (11.5- 12) in addition to the histological alteration may occur because the renal functional maturity

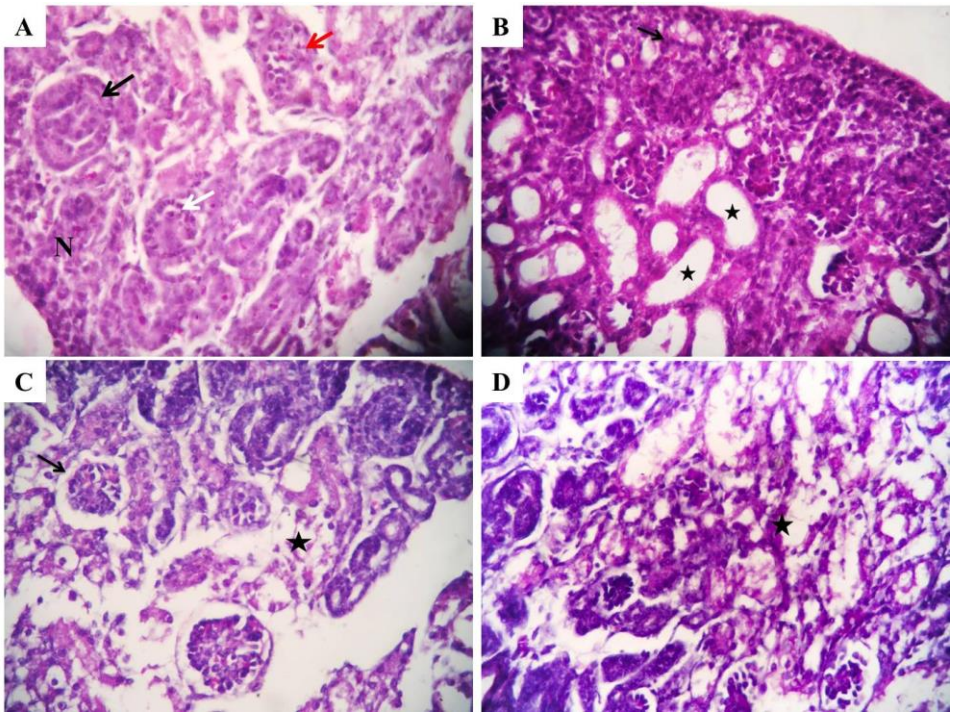

Figure 1: Sections of neonatal kidney exposed to AgNPs during 1st week of gestation period. **A**- Control group shows subcapsular nephrogenic zone (N), comma-shaped body (white arrow), S-shaped body (black arrow), and glomerulus (red arrow). **B**- Low dose group shows cystic tubular dilation (asterisk) with severe degeneration of some renal vesicles (arrow) in subcapsular nephrogenic zone. **C**- Middle dose group shows glomerular tuft shrinkage (black arrow) and focal tubular necrosis (asterisk). **D**- High dose group shows focal tubular necrosis (asterisk) without glomerular alteration. 400x. H&E stain.

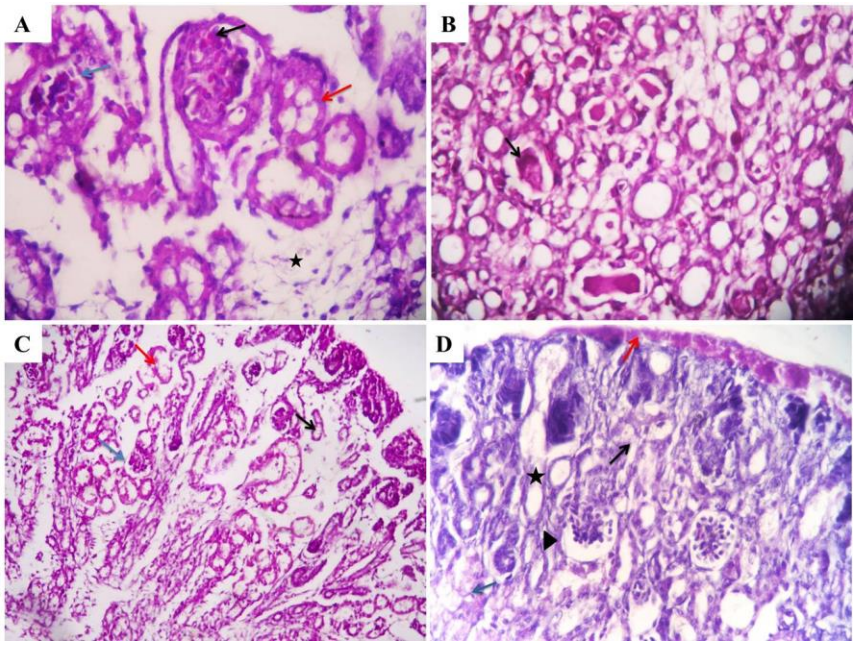

Figure 2: Sections of neonatal kidney exposed to AgNPs during 2nd week of gestation period. A- Low dose group shows disintegrating of immature glomeruli (blue arrow) and vascular congestion of mature glomerular tuft (black arrow) with vacuolar epithelia of tubules (red arrow) 400x. B- Low dose group shows medullar renal tubules with eosinophilic hyaline casts (arrow) 400x. C- Middle dose group shows degenerative changes and tubular necrosis (red arrow), atrophy of surviving tubules (black arrow), and slight distention of Bowman's space (blue arrow). 100x. D- High dose group shows subscapular hemorrhage (red arrow) and disruption of cortical tubules (black arrow), the glomeruli express little changes (head arrow) , and tubular dilation (asterisk) . 400x. H&E stain.

effected via AgNPs after nephron formation complete at E15[23]. These results supported by[22] that explained the importance to determine the stage of feto-placental development when examined the fetotoxicity of nanoparticles as well as the role of visceral yolk sac to transfer these nanoparticles by endocytosis or by the other mechanisms. In addition, the AgNPs in maternal blood circulation may be aggregated and become large in size and their ability to cross the extra-embryonic tissues become more difficult lead to decrease their accumulation in the fetus tissue[1].

Also, the current results showed the developing kidney is more affected by AgNPs during 3rd week of gestation comparing with 2nd week of gestation in a time-depended manner. These results may partly be associated with the fully development of nephrons as it is mentioned previously, and their functional maturation which take place during this week (growth with development stage) and partly because of the placenta exhibits reorganization at GD 18 as preparation to birth or lobar which allow to the AgNPs leakage through it[24,11].

The developing kidney is the main organ for AgNPs accumulation and this was proved by[18] that measured its accumulation in the neonatal kidney after their mothers (pregnant rats) exposed to the high dose of AgNPs. Furthermore, the developing kidney has sensitive windows of exposure to various drugs or chemicals (xenobiotics) which lead to nephrotoxicity, as well as functional maturation defects according to synchronize the developmental time point with the exposure time[24]. Also, the diameter of Ag nanoparticles which transfer across the placenta into embryo tissues in dose-dependent distribution which detected by TEM was approximately 50 nm[11]. Furthermore, recent studies mentioned by[22] that nanoparticles with size approximately 35 -70 nm which were managed in late gestation at high doses, able to pass into fetal tissues across the mice placenta and influence fetal toxicity. These researches support our study where the size of Ag nanoparticles was (35-100) nm, as well as with our results that show the effects of AgNPs on neonatal kidney was more noticeable when pregnant mice exposed to AgNPs during 3rd week of pregnancy in a dose-depended manner comparing with the others treated groups.

Finally, the histopathological alteration in the present study showed these alterations, which mainly occurred after the organogenesis stage by effecting silver nanoparticles on the mature nephrons and their functional structure. The basement membrane of glomeruli and in the mesangium and also of the renal cortical and medullary tubular basement membrane showed greatly accumulation of AgNPs as well as it was detected in the organelles of

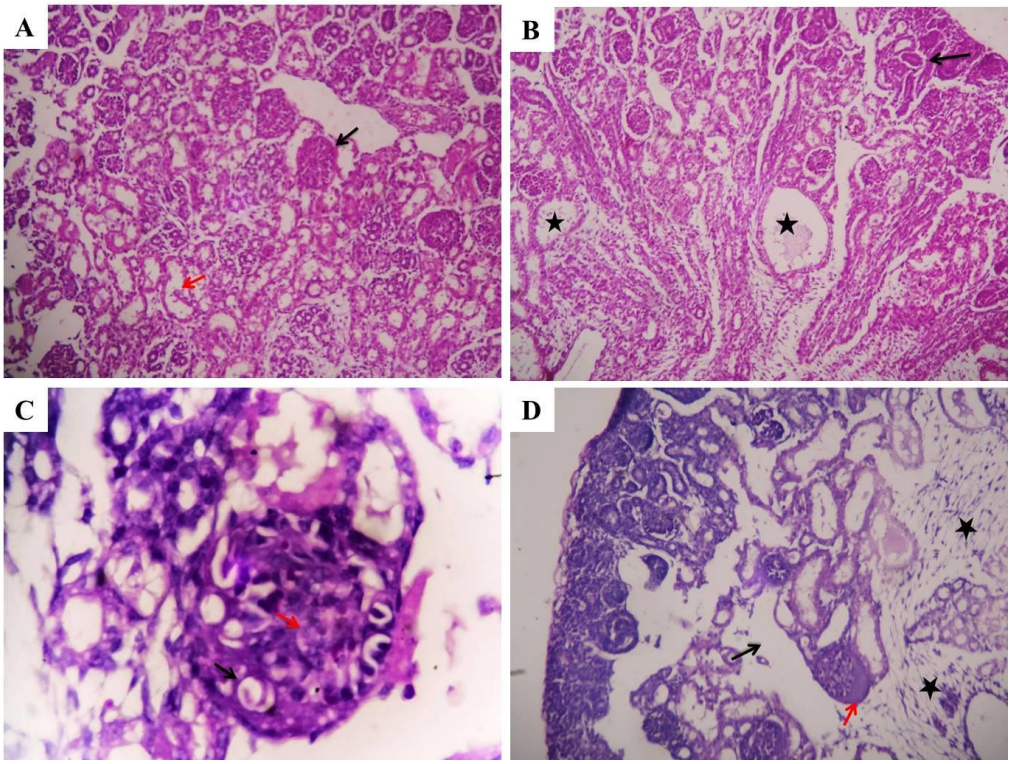

Figure 3: Sections of neonatal kidney exposed to AgNPs during 3rd week of gestation period. A- Low dose group shows renal glomerulus with massive hypercellularity mainly in the deeper part of renal cortex (black arrow) and tubular dilation in renal cortex (red arrow). 400x. B - Middle dose group shows severe atrophy of subcapsular immature tubules (black arrow) and cortical cyst formation (asterisk). 100x. C- High dose group shows glomerular tuft necrosis (red arrow) with vacuolation (black arrow). 400x. D- High dose group shows glomerular structures with dilation of Bowman's space with evidence of crescent formation (red arrow) , severe loss of surrounding parenchyma (black arrow), and the medullar portion replaced by scant loose C.T. tissue (asterisk). 100x. H&E stain.

interstitial cells including cytoplasm and nuclei due to the formation of thiol-silver complexes[A,26]. In addittion, the AgNPs when accumulated in cellular organelles especially the mitochondria caused crystolysis and damage in the inner membrane, the ATP production and the oxidative phosphorylation will be affected, stimulated the cytotoxicity via reactive oxygen species ROS and intracellular oxidative stress lead to induce cell necrosis and apoptosis[27,28,6]. This mechanism explains the appearance of glomerular tuft necrosis and focal tubular necrosis with destruction of interstitial tissue with or without replacement by connective tissue in the neonatal kidney in this present study

The histological changes of neonatal kidney during 2[sd] week repersened by disintegrating of immature glomeruli, tubular necrosis, destruction of dilated tubules, loss of renal parenchyma, subcapsular haemorrhage. These alteration may be asssociated with cells porliferation and apoptosis that regulated by the expression of molecular mediators during nephrogenesis and depletion of fetal antioxidant defense. High levels of ROS have negative effects on the developing embryos during early organogenesis owing to its relation to the antioxidant capacity, lead to increasing in oxidative stress and embryonic damage[29]. In addition, the cell proliferation and apoptosis regulated by various signaling pathways, and the exposure to AgNPs lead to kidney damage by inhibition of the beneficial or normal apoptotic pathway and stimulation of necrotic cells[30].

The appearance of cortical cyst formation in this study may be related to the dysregulation of tubular cells proliferation. Furthermore, the cyst formation in the developing kidneys was explained in a study carried out by[31]. The study showed its formation was related to increased cells proliferation, changes in cell polarity, cell-cell and cell-matrix interactions. Moreover, the glomerular basement membrane damaged severely due to exposure to the xenobiotics, the crescents (cellular extracapillary) can be observed as results of leakage of some plasma proteins together with affected epithelial cells, monocytes and macrophages[32]. This clarifies the appearance of the crescent in the present study during 3[rd] week which synchronic with high dose management. Also,[33] stated that the glomerular necrosis and cortical tubules damage were observed in neonatal Swiss Albino mice when they were exposed to AgNPs with 2.75 -18.5 nm size during (E5 - E15) and additionally, the cortical tubules dysmorphology caused by the nanosilver toxicity lead to increasing water pressure in glomerulus and stimulates glomerular hypertrophy.

CONCLUSIONS

The results suggested that the huge using of AgNPs in different applications during pregnancy has negative effects on developing kidney, as indicated in a time-depended manner and in a dose-depended manner. Comparing of histological changes of neonatal kidney during different developmental time points (gestational stages) showed the degree of these alteration associated with the AgNPs levels in embryo which induce ROS generation and depletion of fetal antioxidant defense leading to embryonic damage, or by it is effect on signalling pathway that regulates nephrogenesis and on its functional maturity. More researches must be carried out to have a better understanding of AgNPs toxicity on fetal development and its ability as a teratogenic agent to induce external and internal abnormalities in the fetus.

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