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In vivo study of silver nanomaterials' toxicity with respect to size

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Abstract

Silver nanoparticles (Ag NPs) are widely used in nanomedicine, pharmaceutical products, industry and other consumer products owing to their unique physiochemical properties with probable potential risk to human health and the ecosystems. The aim of this work was to investigate the in-life morphological effects, biochemical, histological and histochemical alterations that might be induced by variable sizes of Ag NPs in hepatic, renal and testicular tissues with the hypothesis that variable sizes of nano-Ag could induce variable effects in the vital organs. Five groups of adult healthy male mice (BALB/C) were exposed to 35 intraperitoneal injections of Ag NPs (1 mg/kg bw) using five different particle sizes (10, 20, 40, 60 and 100 nm). All mice were subjected to in-life morphometric, biochemical, histological and histochemical analysis. The findings demonstrated that Ag NPs could induce alterations in the average body weight gain, food consumption, water intake and organ indices. In addition, these NPs significantly altered hepatic and renal biomarkers. Moreover, Ag NPs produced ground glass hepatocyte cytoplasm, with mitotic activity, nuclear alterations, degeneration, glycogen depletion and inflammatory cells infiltration in the liver. The kidneys of treated mice exhibited proximal renal tubules degeneration, distal renal tubules regeneration, glomerular shrinkage, Bowman's capsule thickening and interstitial inflammation. The testicular tissues demonstrated spermatocyte sloughing and spermatid giant cell formation. The findings together indicated that Ag NPs could interact with the anatomical structures of the liver, kidney and testis in ways that could induce injury. In addition, the results indicated that smaller Ag NPs posed a greater potential risk than the larger ones, which might be associated with their behaviour, dissolution rate, bioavailability and their probable variable toxicokinetics.

Keywords

Silver nanoparticles, nanotoxicity, liver, kidney, testis

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Introduction

Silver nanoparticles (Ag NPs) have been brought to the market in the last few decades and now constitute considerable investments in medicine, industry, consumer products and other activities owing to their potential antimicrobial features (De Lima et al., 2012; Rai et al., 2012; Xue et al., 2012). Ag NPs have been widely used in the treatment of skin ulcers, burns and wound dressing, together with catheter-related infections and pneumonia (Hermans, 2006; Jain et al., 2009; Lansdown, 2006; Muangman et al., 2006; Park et al., 2019; Qin, 2005; Supp et al., 2005; Wright et al., 2002). Some studies have reported that polyvinyl pyrrolidone-coated Ag NPs demonstrated activity in HIV-1 treatment at low cytotoxic concentrations by preventing the virus from binding to the host cell (Lara et al., 2010). Ag NPs

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are also for coating medical devices such as catheters, synthetic heart valves, blades, surgical needles, and medical cloth to limit bacterial growth (Chopra, 2007; Silver et al., 2006; Turtle, 2012).

Ag NPs have electrical, optical and thermal features and are being used as chemical and biological sensors, and in neural tissues imaging (Ahamed et al., 2010). Several brands of cosmetics, prostheses, toothpaste, dental composites and laundry detergents also include Ag NPs (Jain et al., 2009; Turtle, 2012). In addition, Ag nanofibres are now being used in bioengineering and in water filters to purify contaminated water (Jain and Pradeep, 2005). Moreover, these fine particles have been used in food storage containers and baby toys (Hanson, 2011; Turtle, 2012).

Ag NPs accumulate in vital organs and are considered to be toxic to the liver, endometrium, lungs, spleen and brain (Ahamed et al., 2010; Austin et al., 2011; Johnston et al., 2010; Kim et al., 2010; Riediker et al., 2019; Xue et al., 2012). The nature of Ag NPs, being so small and having a large relative surface area, enables them to enter the cells and interact with organelles and biological molecules (Faraji and Wipf, 2009). Other studies have indicated that the small sizes of these particles and their high surface to volume ratio enable them to disperse easily from the injection site and to enter cells passively, allowing maximum interaction with the biological molecules present (Arora et al., 2012). In addition, Ag NPs size-dependent *in vitro* studies provided evidence of a link between particle size and the degree of toxicity (Pineda et al., 2012; Weldon et al., 2016). Some toxicological studies have concluded that Ag NPs undergo oxidative dissolution in the presence of oxygen, thereby releasing Ag^+ ions (Ma et al., 2012). Other reports showed that this release increased with increasing temperature, time of exposure and concentration and decreased with increasing pH (Liu and Hurt, 2010).

Dermal exposure to Ag NPs may lead to direct penetration into the skin and then entry into the bloodstream. The smaller the particles are, the more they are absorbed and the deeper into the body they can go. Ultrafine particles smaller than 200 nm are no longer phagocytosized and can travel freely in the blood and through the body (Hett, 2004). Also, particles smaller in size than 300 nm can be absorbed by the intestinal epithelium and reach the bloodstream, whereas particles less than 100 nm in size can be absorbed easily by the epithelial tissues and cross the vasculature endothelia (Hett, 2004). In addition, Ag NPs can cross the placenta and reach the foetus.

Ag NPs enter the environment via treatment of food, industrial wastewater and nano-Ag structures and devices (Reijnders, 2006). The evidence from ecotoxicological studies has demonstrated that Ag NPs are toxic to all forms of life and could induce all types of biological and neurological responses (Oberdörster and Kuhlbusch, 2018; Turtle, 2012). These studies reported that the potential risks of Ag NPs exceed those of other metal oxide NPs such as titanium, zinc and iron (Lin et al., 2011).

Nanotoxicological investigations indicated that changes induced by Ag NPs might be associated with their charges and to the release of Ag^+ ions and their interactions with tissue components and biological molecules (Asharani et al., 2009). *In vitro* toxicological reports showed that Ag NPs could produce reactive oxygen species that could induce damage to plasma membranes and intracellular components with probable injury in the mitochondria and cytoskeleton (Chopra, 2007; Parveen et al., 2012; Schrand et al., 2008, 2010). Moreover, Asharani et al. (2009) demonstrated that Ag NPs could induce DNA injury, chromosomal aberrations, cell cycle arrest, reduction of metabolic activity and ATP production in treated cells, thus suggesting mitochondrial dysfunction. Ag NPs can penetrate through cellular barriers, and they increased membrane leakage in mammalian stem cells causing cytotoxicity and genotoxicity and DNA injury (Carlson et al., 2008; Hsin et al., 2008; Kim et al., 2012; Rosenkranz, 2010). Some studies have indicated that Ag NPs could result in inflammation, myocardial infarction, thrombosis and alveolar macrophage cells injury (Asharani et al., 2009; Chen and Schluesener, 2008).

Significant changes were observed in plasma alkaline phosphatase (ALP) activity and blood cholesterol resulted from exposure to 56 nm Ag NPs, while subjection to 42 nm particles significantly elevated the levels of ALP, aspartate transaminase (AST) and alanine transaminase (ALT) (Kim et al., 2010; Park et al., 2010b). On the other hand, no alteration was found on the plasma levels of ALP, AST and ALT following oral administration of Sprague-Dawley rats to different sizes of Ag NPs (5–100 mg/kg) for 14 days (Kulthong et al., 2012). This work found no significant alteration in the hepatic cytochrome P450 (CYP) drug-metabolizing enzymes activity for all tested Ag NPs doses.

Owing to their antibacterial and antifungal properties, Ag NPs are used in various commercial applications in the food industry, household items, biomedicine, clothing, cosmetics, personal hygiene products and water purification. The increased occupational exposure to these nanomaterials (NMs) put persons manufacturing and handling these NMs and their containing products at risk. We began the present work with the hypothesis that variable sizes of Ag NPs induce variable toxicity. Accordingly, the aim of the present study was to investigate the in-life morphological effects, biochemical, microanatomical and histochemical alterations that might be induced by variable sizes of Ag NPs in liver, kidney and testis tissues.

Materials and methods

Experimental animals

Thirty-six adult healthy male mice (BALB/C), weighing 25–28 gm were obtained from the Animal House, Jerash Private University, and randomly distributed into six groups (control group and five treated ones) of six mice each. The animals were housed at controlled temperature (22 ± 2°C), humidity (45 ± 5%) and 12:12 light/dark cycle and maintained in the Nanobiology Unit, Faculty of Science, Jerash Private University. The mice were housed in clear plastic cages ($30 \times 20 \times 20$ cm^3) and provided with standard food and tap water *ad libitum*.

The mice were cared for and treated in accordance with the guide of Canadian Council on Animal Care (Olfert et al., 1993), and the study protocol was approved by the ethical committee of King Abdullah II Fund for Development, Jordan (reference number 2-2017).

Silver Nanoparticles

Ag NPs (10, 20, 40, 60 and 100 nm) purchased from Sigma-Aldrich (St. Louis, Missouri, USA) were used in the present work. Each batch was accompanied with a certificate from the manufacturer concerning the physicochemical characterization such as the average particle size, surface area, ultraviolet–visible spectrum, concentration, density.

Experimental protocol

After a 1 week acclimation period, the tested groups were injected by the intraperitoneal (ip) route with a daily single dose (1 mg/kg bw) of Ag NPs using five different sized Ag NPs (10, 20, 40, 60 and 100 nm) in diameter for 35 injections while the control mice were exposed to NPs vehicle (aqueous citrate buffer, pH = 6.2). A fresh dispersion of each of the five different sized Ag NPs was disaggregated by ultrasonication after dilution with the vehicle NPs at 37°C immediately before use. A solution of each ultrasonication was prepared so that the dose could be determined of 1 ml via ip route.

In-life observations

Daily observation throughout the study was made for mortality, general well-being, reaction to Ag NPs treatment and behaviour patterns. The ratio of food consumption (g) to mouse body weight (g) after treatment with different sizes of Ag NPs was calculated weekly for each group. In addition, the weekly ratio of water intake to mouse body weight (ml/g) after treatment with different sizes of Ag NPs was also measured.

The body weight of the mice was monitored at the beginning of the exposure to Ag NPs and subsequently every week and once more on the day of termination and necropsy. Liver, kidneys and testes from each mouse were removed carefully, weighed in grams (absolute organ weight) and the relative organ weight to body weight for each mouse was calculated according to Aniagu et al. (2005).

The amount of change on the relative ratio of the organ weight to body weight and the liver, kidneys and tests organ index for all mice groups under study was calculated according to Sardari et al. (2012) as follows

$$\text{Organ index} = \frac{\text{Average weight of the treated organ/average weight of the treated mice}}{\text{Average weight of the control organ/average weight of the control mice}}$$

Biochemical analysis

Forty-eight hours after the last Ag NPs injection, all mice under study were anaesthetized with an ip injection of pentobarbital (70 mg/kg). Each animal was euthanized by exsanguinations via blood drawing from the orbital sinus and cardiac puncture. Plasma samples in heparin sodium blood tubes were separated by centrifugation (Thermo Fisher Scientific centrifuge, Waltham, Massachusetts, USA) for 6 min at 1812 x g and subjected to the following biochemical tests: ALT, AST, ALP, albumin, total proteins, creatinine, urea, uric acid, total bilirubin, triglyceride and total cholesterol. Analysis was carried out by using an Auto-chemistry Analyser-BS 300-2772121272 (Mindray Bio-Medical Electronics Co., Shenzhen, China).

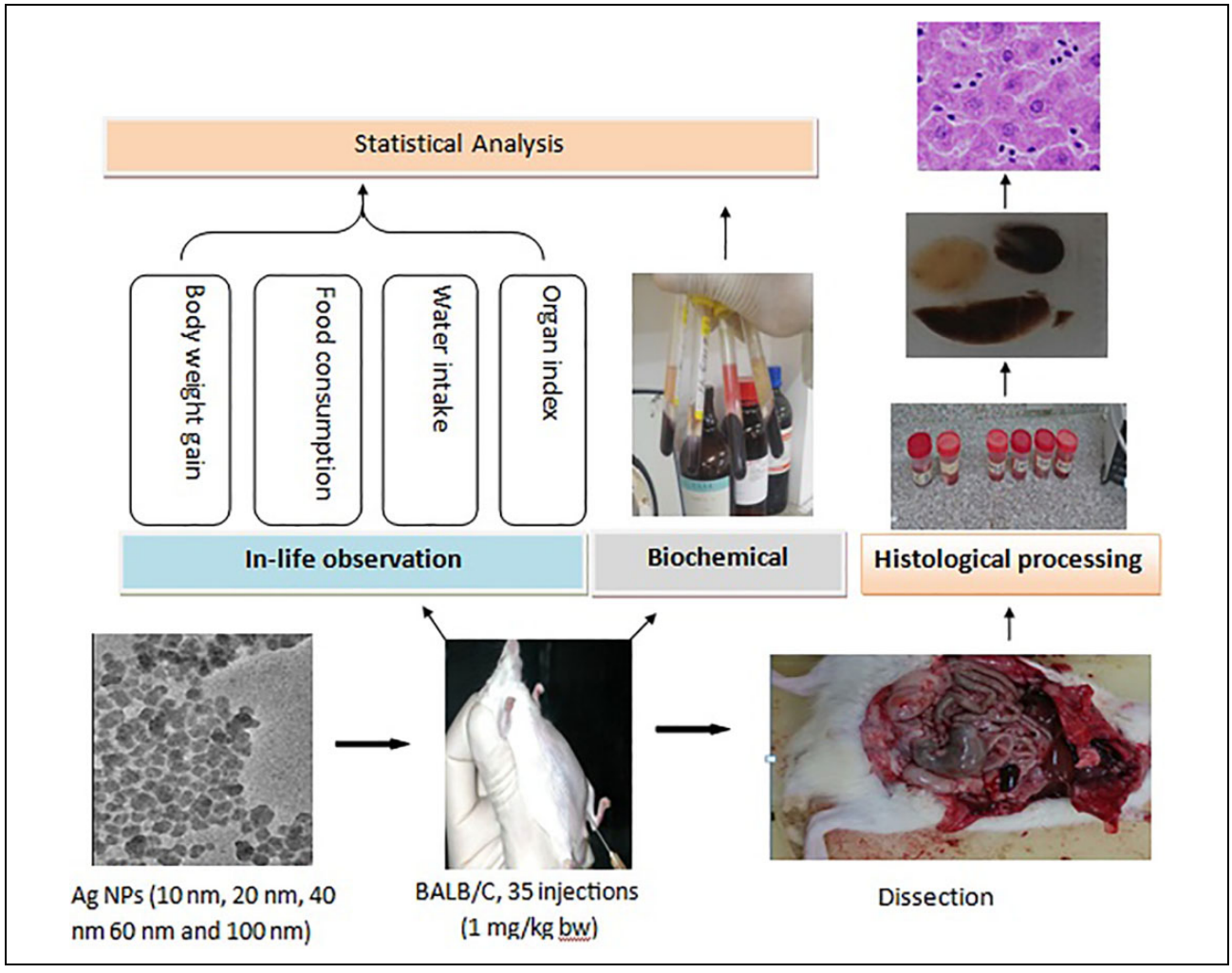

Figure 1. Graphic design of experiments and analysis conducted by the present study.

Histological and histochemical processing

Hepatic, renal and testicular biopsies were taken from each mouse under study including small portions of median lobe of the liver, right kidney (cortex and medulla) and the left testis. All tissues for histological examination were processed for fixation in buffered neutral formalin, dehydration by ascending concentrations of ethanol, clearing by xylene, wax impregnation by molten paraffin wax (melting point 58°C), embedding, blocking out and sectioning at 4–5 µm. Tissue processing was carried out by automatic Tissue Processor (Thermo Shandon Company, Waltham, Massachusetts, USA). Paraffin sections were stained using the following conventional histological and histochemical techniques: haematoxylin and eosin, Mallory trichrome, reticulin stain, periodic acid–Schiff stain and Perl's reaction. Stained histological sections of the liver, kidneys and testes of control and Ag NPs-treated mice were subjected to histopathological examination.

Figure 1 summarizes the experiments and techniques in the present study.

Statistical analysis

One-way analysis of variance test was used as a statistical tool to compare the in-life observations and biochemical alterations between the control group and the Ag NPs-treated groups. Statistically significant changes compared with the control group were indicated as $*p < 0.05$.

Results and discussion

In-life alterations

Change in body weight gain. All mice administered Ag NPs demonstrated a decline in their average body

weight gain in comparison with the control group (Table 1). This change was more significant ($p < 0.05$) in mice administered 10 or 20 nm Ag NPs. This suggests that the smaller size Ag NPs have a greater potential to induce toxicity, possibly by increased oxidative stress, than the larger sizes.

Food consumption. All Ag NPs-treated mice in the present work demonstrated a significant ($p < 0.05$) decline in food consumption compared to the control group (Table 1).

Water intake. Mice administered nano-Ag showed increased water intake (ml) per gram of body weight. This alteration was significant ($p < 0.05$) in all mice that received 60 nm or lesser sizes of Ag NPs (Table 1).

Gall bladder ballooning. Mice exposed to 20 nm Ag NPs demonstrated cholestasis with ballooning of the gall bladder. This injury may indicate hepatotoxicity related to an elevation of ALP and hepatocytes necrosis (Kaplowiz, 2004; Singh et al., 2011).

Organ index value. The findings of the present work showed that mice exposed to 10 nm Ag NPs demonstrated a decline in the liver index (0.91), kidneys index (0.92) and testes index (0.94) values in comparison with the control mice indices (1.0) as seen in Table 1. This alteration was less prominent in mice exposed to larger particles. This result is in line with the findings of other studies (Sardari et al., 2012). The organ index alteration is a sensitive parameter for toxicity affecting the metabolism (Bailey et al., 2004). The results of the present investigation showed that the relative ratio of the organ weight to the body weight for the liver was decreased significantly ($p < 0.05$) only in the mice treated with 10 nm Ag NPs in comparison with those exposed to particles of larger sizes. In addition, Ag NPs exposure decreased significantly the total kidneys index. The percentage absolute kidney weights were significantly reduced in mice that received 10 and 20 nm Ag NPs in comparison with mice subjected to particle sizes of 40 nm or more. The testes index was less affected by Ag NPs exposure in comparison with that of the liver and the kidneys. Mice exposed to 10 or 20 nm Ag NPs demonstrated a slight decrease in testes index value while those subjected to 40, 60 and 100 nm were almost not affected (Table 1).

Biochemical alterations

Significant ($p < 0.05$) elevation of AST, ALT and ALP was recorded in plasma of all Ag NPs-treated mice with significant elevation in those treated with 10, 20 or 60 nm particles than for those exposed to larger particles (Table 2). The liver is the site of detoxification and performs more than 500 metabolic functions (Singh et al., 2011). Plasma levels of the liver function enzymes such as ALT, AST and ALP are used as indicators for hepatocytes injury (Singh et al., 2011). Both ALT and AST are used as a biomarker of the health of hepatic tissues. While AST is more wide spread than ALT in the cardiac and skletal muscles, it can be indicatve in the pathology diagnosis of these tissue (Singh et al., 2011). The findings of the present work agree with other investigations (e.g. Monfared and Soltani, 2013). A case study indicated that a Ag-coated wound dressing resulted in elevated levels of plasma liver enzymes (Trop et al., 2006). The activity of ALP ws seen in all tissues but was mainly anchored in the hepatocytes and biliary duct epithelia and becamemore active as a result of hepatocellular injury and bile duct obstruction (Singh et al., 2011). The elevation of ALP resulting from exposure to Ag NPs with the cholestasis of treated mice, as seen in the present study, may reveal bile duct sensitivity affecting hepatocytes integrity. Some studies have reported that ALP elevation is related to drugs and chemicals that cause cholestasis (Preussner, 1998; Wright and Vandenberg, 2007).

The findings of the present study indicated that total protein levels were decreased in all mice treated with Ag NPs, with the greater decrease noted in those exposed to 10 or 20 nm particles (Table 2). Albumin level was also decreased significantly ($p < 0.05$) in groups of mice subjected to Ag NPs. Total protein concentration is often reduced slightly during hepatocellular damage (Singh et al., 2011). The findings of the present work are consistent with those of Monfared and Soltani (2013) who reported that Ag NPs induced significant decrease in total protein level in the blood of rainbow trout (*Oncorhynchus mykiss*).

Bilirubin is a breakdown product of haemoglobin in red blood cells (Singh et al., 2011). An elevation of total bilirubin levels was also demonstrated by mice subjected to Ag NPs. Total bilirubin levels were elevated significantly ($p < 0.05$) in those mice exposed to 10 and 20 nm and 40 nm Ag NPs, whereas a lesser elevation was observed in mice subjected to 60 nm

Table 1. Morphometric alterations induced by variable sizes of Ag NPs.[a]

Group	Starting and final food consumption (food (g)/ g (bw))	Food consumption decline (%)	Starting and final water intake (water (ml)/g (bw))	Water intake increase (%)	Starting and final body weight	Body weight gain (%)	Liver index	Kidneys index	Testes index
Control mice	2.17–2.09	3.69% ± 1	0.62–0.59	−4.84% ± 0.4	27.4 ± 0.9–30.5 ± 3.2	11.31% ± 2.1	1.0 ± 0.13	1.0 ± 0.1	1.0 ± 0.12
Mice received 10 nm Ag NPs	2.23–1.82	18.38% ± 3[b]	0.66–0.71	+5.58% ± 0.4[b]	27.8 ± 1.6–29.1 ± 2.9	4.68% ± 0.7[b]	0.91 ± 0.1[b]	0.92 ± 0.1[b]	0.94 ± 0.13
Mice received 20 nm Ag NPs	2.19–1.86	15.07% ± 2[b]	0.64–0.75	+17.19% ± 2[b]	26.9 ± 2.3–28.5 ± 1.8	5.96% ± 0.9[b]	0.95 ± 0.12	0.92 ± 0.13[b]	0.96 ± 0.12
Mice received 40 nm Ag NPs	2.27–2.08	8.37% ± 0.1[b]	0.61–0.68	+11.84% ± 2[b]	27.2 ± 1.2–29.3 ± 3.1	7.72% ± 1.1	0.96 ± 0.15	0.96 ± 0.14	0.99 ± 0.15
Mice received 60 nm Ag NPs	2.16–1.93	10.64% ± 1.2[b]	0.59–0.65	+10.17% ± 1.5[b]	26.8 ± 0.9–29.7 ± 2.5	10.82% ± 1.4	0.95 ± 0.1	0.94 ± 0.12	1.02 ± 0.11
Mice received 100 nm Ag NPs	2.14–1.97	7.94% ± 1[b]	0.63–0.61	−3.17% ± 0.5	26.1 ± 1.5–28.1 ± 4.3	7.66% ± 1.8	0.96 ± 0.12	0.98 ± 0.15	1.03 ± 0.10

Ag NP: silver nanoparticle; ANOVA: analysis of variance.

[a]The results are represented as mean ± standard deviation.

[b]$p < 0.05$: statistical difference in comparison to the control and using one-way ANOVA test.

Table 2. Biochemical alterations induced by variable sizes of Ag NPs.[a]

Group	ALT (U/l)	AST (U/l)	ALP (mg/dl)	Total protein (g/l)	Albumin (mg/dl)	Creatinine (mg/dl)	Urea (mmol/l)	Uric acid (mg/dl)	Total bilirubin (mg/dl)	Triglyceride (mg/dl)	Total cholesterol (mg/dl)
Control mice	42.25 ± 11	170.38 ± 28	43.49 ± 8	48.23 ± 0.7	179.72 ± 26	1.15 ± 0.2	8.73 ± 1.3	5.48 ± 1	16.75 ± 3.1	81.16 ± 17	107.09 ± 15
Mice received 10 nm Ag NPs	137.11 ± 23[b]	313.61 ± 39[b]	71.82 ± 12[b]	43.42 ± 0.3[b]	115.94 ± 21[b]	1.97 ± 0.2[b]	7.55 ± 1.1[b]	8.31 ± 1[b]	23.39 ± 2.6[b]	196.14 ± 25[b]	98.12 ± 13
Mice received 20 nm Ag NPs	128.17 ± 26[b]	296.25 ± 35[b]	92.76 ± 15[b]	39.97 ± 0.7[b]	121.11 ± 20[b]	2.09 ± 0.2[b]	9.03 ± 0.9	6.77 ± 0.9[b]	26.11 ± 3[b]	202.37 ± 23[b]	93.86 ± 17
Mice received 40 nm Ag NPs	119.26 ± 28[b]	215.36 ± 24[b]	68.44 ± 11[b]	41.05 ± 0.6[b]	123.51 ± 18[b]	2.12 ± 0.2[b]	7.88 ± 2.1	7.28 ± 1[b]	38.71 ± 4.2[b]	213.28 ± 31[b]	88.67 ± 18
Mice received 60 nm Ag NPs	63.20 ± 19	237.61 ± 41[b]	77.23 ± 14[b]	47.33 ± 1	119.82 ± 24[b]	1.85 ± 0.2[b]	7.94 ± 1.4	6.95 ± 0.8[b]	21.55 ± 3.3	190.51 ± 29[b]	109.11 ± 16
Mice received 100 nm Ag NPs	56.82 ± 18	205.17 ± 31	59.61 ± 12	50.28 ± 1.1	131.08 ± 16[b]	1.73 ± 0.2[b]	8.10 ± 1	6.04 ± 1.1	18.25 ± 2.9	176.01 ± 30[b]	101.57 ± 17

Ag NP: silver nanoparticle; ANOVA: analysis of variance; ALP: alkaline phosphatase; AST: aspartate transaminase; ALT: and alanine transaminase.

[a]The results are represented as mean ± standard deviation.

[b]$p < 0.05$: statistical difference in comparison to the control and using one-way ANOVA test.

particles and with almost no alterations in those treated with 100 nm particles. This pattern suggests hepatobiliary injury by the smaller particles and consequent insufficient ability of the liver to remove the waste metabolic products, including bilirubin.

Triglycerides were significantly ($p < 0.05$) increased and cholesterol levels were decreased in all groups exposed to all variable sizes of Ag NPs under study (Table 2). Previous studies revealed that a low level of cholesterol was related to hepatocellular injury (Singh et al., 2011), while elevation of triglycerides levels was seen in some hepatic and renal diseases (Blanton, 2009; Reddy and Rao, 2006).

Blood concentration of creatinine and urea are used to determine kidney performance. Creatinine plasma levels were elevated significantly in mice exposed to 60 nm Ag NPs or lesser sizes in comparison with the control mice (Table 2). Some reports demonstrated that plasma total proteins are often reduced as a result of liver injury (Thapa and Walia, 2007). In addition, significant ($p < 0.05$) elevation in the levels of uric acid was observed in the plasma of mice subjected to 60 nm Ag NPs or lesser sizes while the levels of urea were decreased significantly ($p < 0.05$) in mice exposed to 10 nm Ag NPs (Table 2). The increased creatinine levels due to Ag NPs exposure might indicate a deficiency in the filtering function of the kidney.

These biochemical alterations may indicate that silver nanomaterials (Ag NMs) could cause injury to the liver, kidney and other vital organs. These findings are in accord with those of other investigators (Monfared and Soltani, 2013; Nel et al., 2009; Seki et al., 2004) who reported that Ag NPs lowered the level of total proteins.

Hepatic histological alterations

The following histological alterations were demonstrated by Ag NPs in the hepatic tissues of mice under study in comparison with the control group.

Control mice. The lobular architecture and zonal accentuation in the liver of the control-group mice were well preserved and intact and demonstrated normal hepatocytes, sinusoids and hepatic portal triads (Figure 2(a)). No histological alterations were seen in the liver of the control mice with no changes in the hepatocytes content of glycogen.

Type II ground glass cytoplasm appearance. Some of degenerative hepatocytes in clusters showed eosinophilic granular glassy cytoplasm (Figure 2(b)). Several reports have indicated that the formation of this alteration is induced by chronic exposure to toxicants and drugs (Su et al., 2008). These alterations were seen mainly in the hepatocytes of mice exposed to 10 nm Ag NPs and to a lesser extent in the cytoplasm of those that received 20 nm particles.

Apoptosis. Mice exposed to 10 or 20 nm Ag NPs exhibited apoptotic hepatocytes (Figure 2(c)). Sardari et al. (2012) reported that exposure to 70 nm Ag NPs for 30 days induced apoptosis. This alteration is induced by toxicants that attack the cell organelles and might be related to intercellular stress (Singh et al., 2011).

Mitotic activity induction. Mitotic activity was more frequently demonstrated by the hepatocytes of mice administered 10 nm Ag NPs and to a lesser extent in those that received 40 and 60 nm Ag NPs (Figure 2(d)). Some hepatocytes demonstrated different stages of mitosis. Mitotic figures were not seen in the hepatic tissues of mice exposed to 100 nm Ag NPs.

Inflammatory cell infiltration. Inflammatory cells were seen infiltrating the hepatic tissues, mainly in the degenerative hepatocytes of mice treated Ag NMs (Figure 2(e)). Previous studies reported similar findings resulted from chronic exposure to 56 nm Ag NPs (Kim et al., 2010).

Nuclear abnormality. Binucleation, anisokaryosis, karyorrhexis nuclear vesiculation, karyolysis and nuclear envelope irregularity were seen in the hepatocytes of mice treated with 10 and 20 nm Ag NPs and to a lesser extent in the liver of mice administered larger particles (Figure 3(a)). Occasional binucleation was observed in the hepatic tissue of the control mice and those exposed to 100 nm particles.

Necrosis. Necrotic degeneration of hepatocytes was mainly demonstrated in the pericentral and midzonal hepatocytes (Figure 3(b)). This alteration was mainly seen in the hepatic tissue of mice exposed to 10 or 20 nm Ag NPs. Cellular necrosis is a consequence of protein synthesis reduction owing to the cessation of RNA synthesis and indicates recent tissue injury (Vetter, 1998). Some reports have indicated that chronic exposure to Ag NPs could induce hepatic necrosis (Ji et al., 2007).

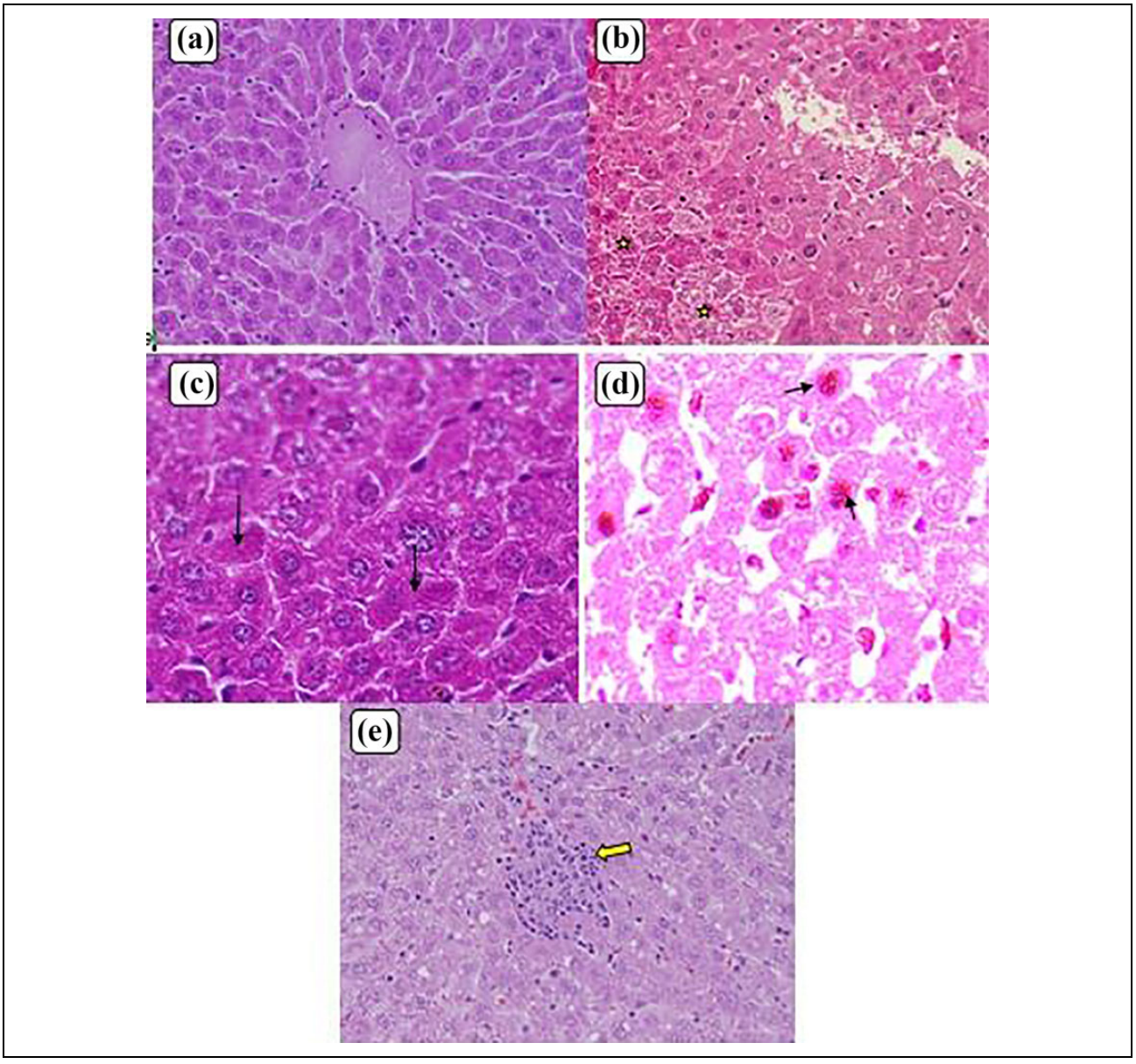

Figure 2. Histological alterations induced by different sizes of Ag NPs. (a) Control liver (270×). Note the well preserved and kept intact hepatic lobules, zonal accentuation and normal hepatocytes. (b) Ground glass cytoplasm hepatocytes (stars), 10 nm Ag NPs (270×). (c) Apoptotic hepatocyte (arrows), 10 nm Ag NPs (540×). (d) Mitotic activity (arrows), 10 nm Ag NPs (270×). Note the prominent mitotic prophase stage. (e) Inflammatory cells inflammation (yellow arrow), 20 nm Ag NPs (270×). Ag NP: silver nanoparticle.

Megakaryocytes induction. Megakaryocytes were observed in the liver of mice exposed to 10 nm particles and to a lesser extent in mice that received 20 nm particles (Figure 3(c)). This alteration may indicate an oxidative stress on the bone marrow and vascular endothelial traumatic injury induced by Ag NMs. Megakaryocytes play an essential role in the synthesis of blood platelets and may indicate stress on haematopoiesis (Pacheco et al., 2002).

Sinusoidal widening. Dilatation of the sinusoids was observed mainly in the hepatic tissues of mice exposed to 10 or 20 nm Ag NM particles (Figure 3(d)). This hepatic change might be associated with oxidative stress in the hepatic tissue induced by these particles. Moreover, this alteration might be the result of sinusoids endothelia injury.

Kupffer cells hyperplasia. Mice subjected to Ag NPs exhibited enlarged and prominent Kupffer cells

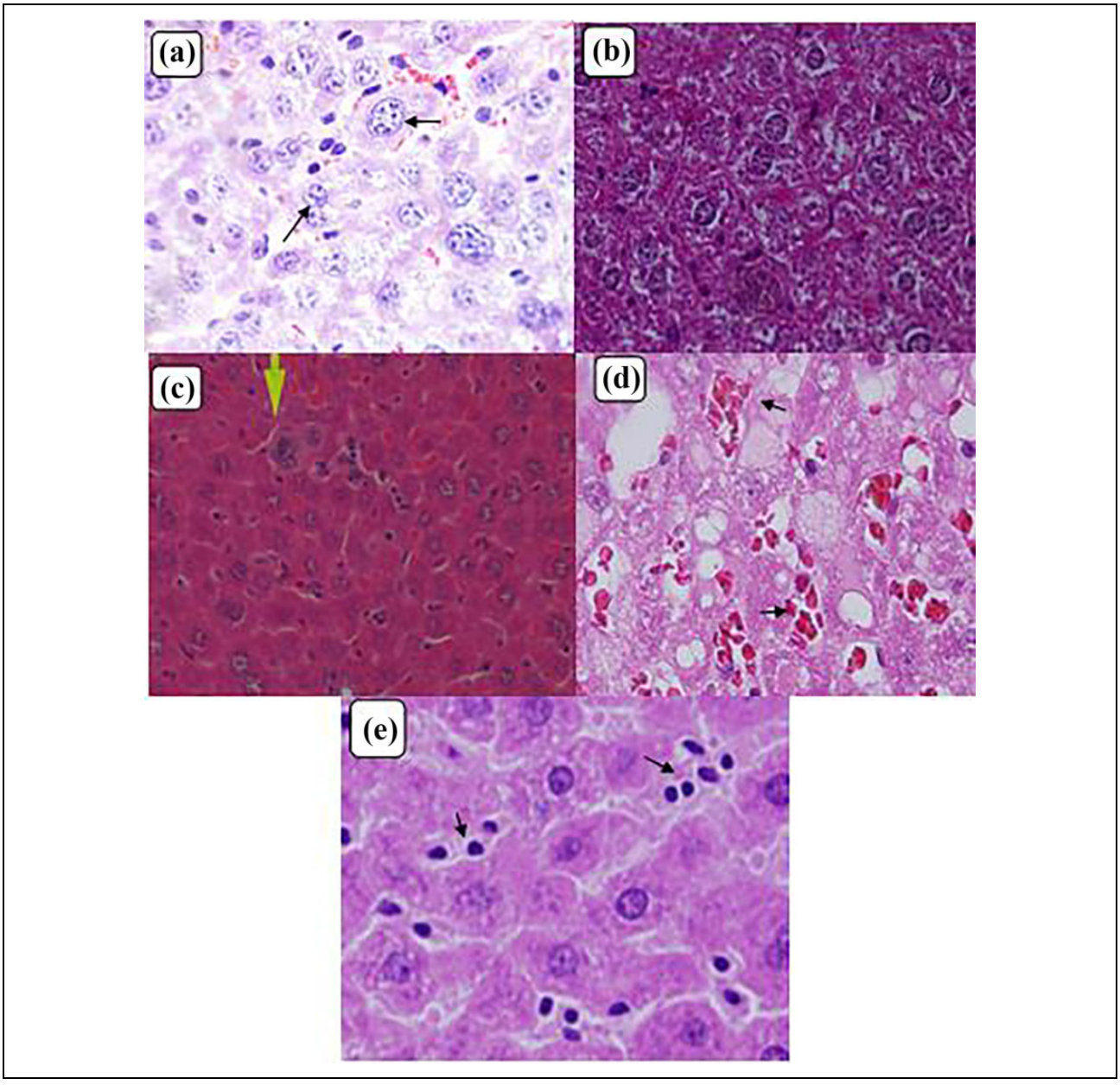

Figure 3. Hepatic histological alterations induced by different sizes of Ag NPs: (a) anisokaryosis and nuclear irregularity (arrows), 40 nm Ag NPs, Mallory trichrome (540×); (b) hepatocytes necrosis, 20 nm Ag NPs (270×); (c) megakaryocytes (arrow), 20 nm Ag NPs (270×); (d) sinusoidal dilatation (arrows), 20 nm Ag NPs (540×); and (e) Kupffer cell hyperplasia (arrows), 60 nm Ag NPs (540×). Ag NP: silver nanoparticle.

(Figure 3(e)). This change was observed mainly in the liver of mice exposed to 20 nm Ag NPs. Studies showed that Ag NMs could increase the phagocytic activity by activating Kupffer cells as an essential tool of hepatic detoxification (Neyrinck, 2004).

Cytoplasmic vacuolation. Swelling and vacuolation of hepatocytes was demonstrated in the hepatic tissue of mice subjected to 10 or 20 nm Ag NPs and to a lesser extent in the liver of mice subjected to 40 or 60 nm Ag NPs (Figure 3(d)). This injury was not seen in the control-group liver and the hepatic tissue of mice that received 100 nm particles.

Hepatic portal space fibrosis. Occasional fibrosis was seen in the hepatic portal space of some mice subjected to 20 nm particles. This finding is consistent with that reported by other investigations (Almansour et al., 2016a,b).

Overall findings of the present work indicate that the liver is a target organ for Ag NMs as indicated in Table 3. This finding is in line with the results of other studies (Austin et al., 2011; Kim et al., 2007, 2010). The liver receives high blood flow causing its tissues to be subject to higher exposure to smaller NPs than the larger ones (Olivier, 2005).

Impact of Ag NPs on hepatocytes glycogen storage

Partial depletion of the glycogen content of hepatocytes was observed in the hepatic tissues of mice exposed to 10 nm Ag NMs and to a lesser extent on mice treated with 20 nm particles (Figure 4(a) to (d)). The depletion was mainly observed in the glassy cytoplasmic hepatocytes. The liver of mice administered 40 and 60 nm particles was less affected, with almost no glycogen depletion in the hepatocytes of animals exposed to 100 nm particles. These findings may indicate that Ag NPs could affect glycogenesis or/and glycolysis.

No hemosiderin pigments precipitation or reticular fibres alterations were detected in the hepatic tissues of all groups of mice treated by Ag NPs.

Alterations induced in the renal tissues

Control kidney. Both cortex and medulla of all control mice kidneys demonstrated normal glomeruli together with normal proximal and distal renal tubules. Loops of Henle and the collecting tubules also appeared normal (Figure 5(a)). Compared to the control renal tissues, the following renal histological alterations were observed in the kidneys of mice exposed to variable sizes of Ag NPs as in Figure 5.

Renal tubules regeneration. The kidneys of the mice injected with 10 nm sized particles demonstrated mild renal tubules regeneration (Figure 5(b)). This change was seen more clearly in the proximal convoluted tubules than the distal ones. This increase of cell turnover in renal tubules wall may indicate a process of renal tissue repair in response to injury induced by Ag NMs.

Inflammatory cells inflammation. Mice treated with 20 nm Ag NPs demonstrated prominent inflammatory cells inflammation in the renal interstitial tissues (Figure 5(c)). Other investigations have reported infiltration of inflammatory cells in the renal cortical tissue of mice administeredorally to 1 mg/kg of 42 nm Ag NPs for 1 month (Park et al., 2010a).

Glomerular atrophy. This injury was seen in the renal tissue of mice that received 10 nm Ag NPs. The shrunken glomerular tuft occupied a small portion of Bowman's space in comparison with the control renal tissues (Figure 5(d)).

Tubular basophilia. The animals subjected to 10 nm Ag NPs demonstrated occasional basophilia (Figure 5(e)). This alteration was previously reported in the kidney of rats received 56 nm Ag NPs for 90 days (Kim et al., 2010).

Tubular necrosis. This type of damage was seen in the renal tissues of mice exposed to 10 or 20 nm Ag particles (Figure 5(f)). Some necrotic renal tubules showed thinning or absence of renal cells brush borders.

Fibrocytes proliferation. Fibrocytes proliferation was seen occasionally in the renal intertubular interstitial tissues of mice that received 10 nm Ag NPs (Figure 5(g)).

Bowman's capsule thickening. The kidneys of mice exposed to 10 nm Ag NPs exhibited foci of Bowman's capsule thickening (Figure 5(h)). This injury was seen but to a lesser extent in the renal tissues of mice subjected to 20 nm particles.

Hyaline casts formation. This injury was visible in the distal convoluted tubules but not in the proximal tubules. This alteration was more prominent in the kidneys of mice that received 10 or 20 nm particles and to lesser extents in those exposed to 40 nm ones. The renal tubules lumina of animals subjected to larger particles did not demonstrate hyaline casts.

The kidneys of mice treated with 60 nm Ag materials demonstrated little evidence of histological abnormalities in the renal tubules, glomeruli and renal interstitial tissues.

The renal alterations induced by Ag NPs, as summarized in Table 3, were more prominent in the cortical renal tissues than the injuries seen in the medullary portions. This might be because the blood flow to the cortex was more loaded with Ag NPs than the blood flowed in the medulla. In addition, the results of the present work revealed that the proximal

Table 3. Histological alterations induced by variable sizes of Ag NPs.[a]

	Hepatic histological alterations											Renal histological alterations							Testicular histological alterations			
	Parameters																					
Group	1	2	3	4	5	6	7	8	9	10	11	11	13	14	15	16	17	18	19	20	21	22
Control mice	−	−	−	−	−		−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	
Mice received 10 nm Ag NPs	++	+	++	+	+	++	+	++	±	+	+	++	±	±	+±	++	++	+	+±	+	++	+
Mice received 20 nm Ag NPs	+	+±	++	+	++	++	±	++	+±	+±	+	±	++	±	−	±	+	++	+	±	+	−
Mice received 40 nm Ag NPs	−	−	+±	+±	+	−	+	±	+	−	+	−	−	−	−	±	+	±	−	±	−	±
Mice received 60 nm Ag NPs	−	−	+	±	±	+	−	±	−	+	−	−	±	−	−	−	−	−	±	−	−	−
Mice received 100 nm Ag NPs	−	−	±	−	±	±	−	−	−	−	−	−	±	−	−	−	−	−	−	−	−	−

Ag NP: silver nanoparticle.

[a]Key: 1, Ground glass cytoplasm; 2, apoptosis; 3, necrosis;4, mitotic activity; 5, hepatic inflammatory cells infiltration; 6, nuclear alterations; 7, megakaryocytes induction; 8, sinusoidal dilatation; 9, Kupffer cells hyperplasia; 10, cytoplasmic vacuolation; 11, hepatic portal space fibrosis, 12, renal tubules regeneration; 13, renal inflammatory cells infiltration; 14, glomerular atrophy; 15, tubular basophilia; 16, fibrocytes proliferation; 17, Bowman's capsule thickening; 18, renal hyaline cast formation; 19, seminiferous tubules necrosis; 20, intertubular oedema; 21, spermatocytes sloughing; 22, spermatid giant cells formation. Alteration degree: −, absent; ±, occasional; +, moderate; ++, strong.

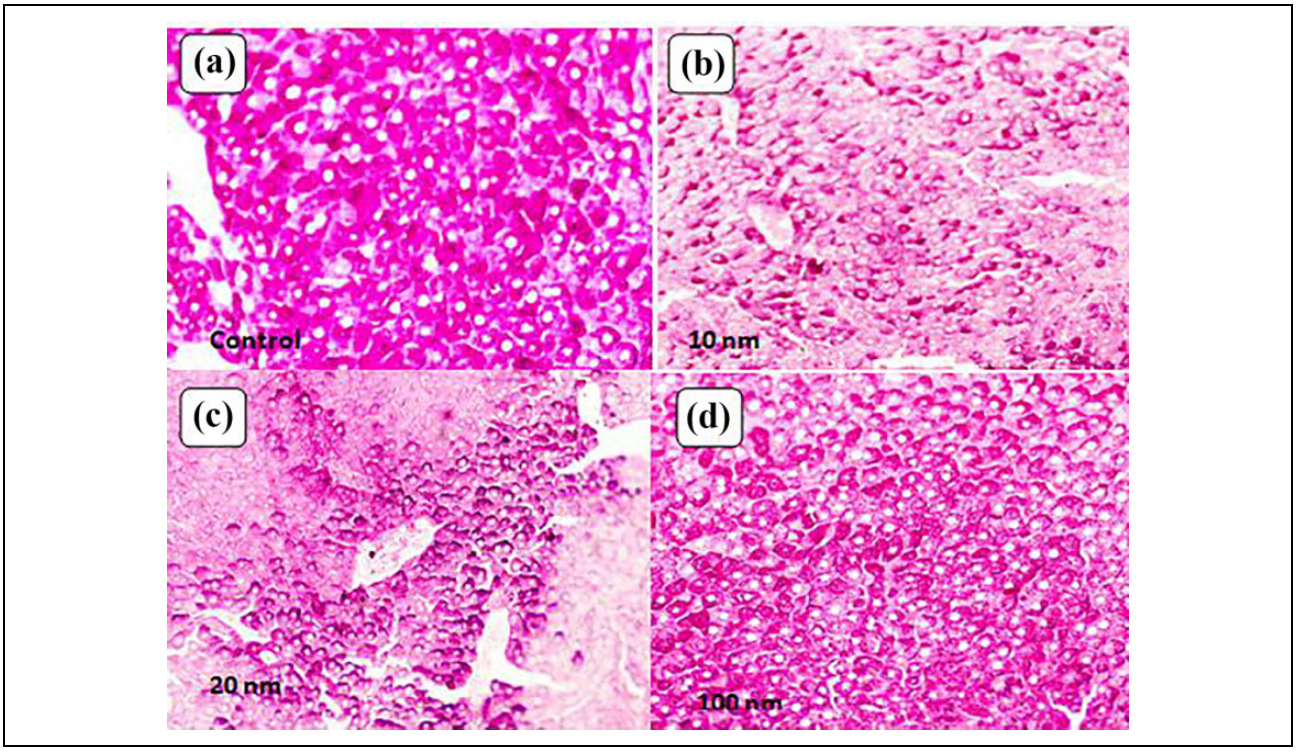

Figure 4. Glycogen content in the liver of (a) control mouse (270×), (b) mouse exposed to 10 nm Ag NPs (270×), (c) mouse exposed to 20 nm Ag NPs (270×) and (d) mouse exposed to 100 nm Ag NPs (270×). Note that exposure to 10 nm particles induced almost complete depletion while the glycogen of mice exposed to 100 nm was almost not affected. Ag NP: silver nanoparticle.

convoluted tubules were more damaged than the distal convoluted ones. These findings are consistent with those seen in previous studies (Sardari et al., 2012). This injury might result from greater viability of Ag NMs in the proximal tubules that function mainly as primary sites of filtrate reabsorption. Moreover, the findings of the present work indicated that Ag NPs could induce damage in the glomeruli and thickened their basement membranes. These, together with the gathering of hyaline casts in the proximal renal tubules, may indicate subsequent glomerulonephritis resulted from Ag NPs toxification affecting the ion pump across the renal tissues.

Alterations induced in the testicular tissues

Control testes. Examination of testicular tissues in the control mice demonstrated normal, intact, seminiferous tubules at various stages of spermatogenesis together with normal testicular interstitial tissues (Figure 6(a)). Exposure to variable Ag NPs induced the following changes in the testicular tissues of mice under study as shown in Figure 6.

Seminiferous tubular degeneration. In comparison with the control animals, mice exposed to 10 nm Ag NPs showed seminiferous tubules 'ghost degeneration', with spermatocytes vacuolation (Figure 6(b)). This change was demonstrated to a lesser extent in the tissues of mice treated with 20 nm Ag NPs, was barley observable in mice that received 40 and 60 nm Ag NPs, and it was totally absent in the testicular tissue of mice exposed to 100 nm particles.

Intertubular oedema. Intertubular oedema was seen in the testicular interstitial tissues of mice that received 10 nm particles and to a lesser extent in those exposed to 20 nm Ag NPs (Figure 6(c)).

Spermatocytes sloughing. Mice administered 10 and 20 nm Ag NPs exhibited desquamation of spermatocytes. The detachment of spermatids and their accumulation with the desquamated spermatocytes together with spermatids were detected in some seminiferous tubules lumina (Figure 6(d)).

Spermatid giant cells formation. Spermatid giant cells appeared in the lumina of some seminiferous tubules

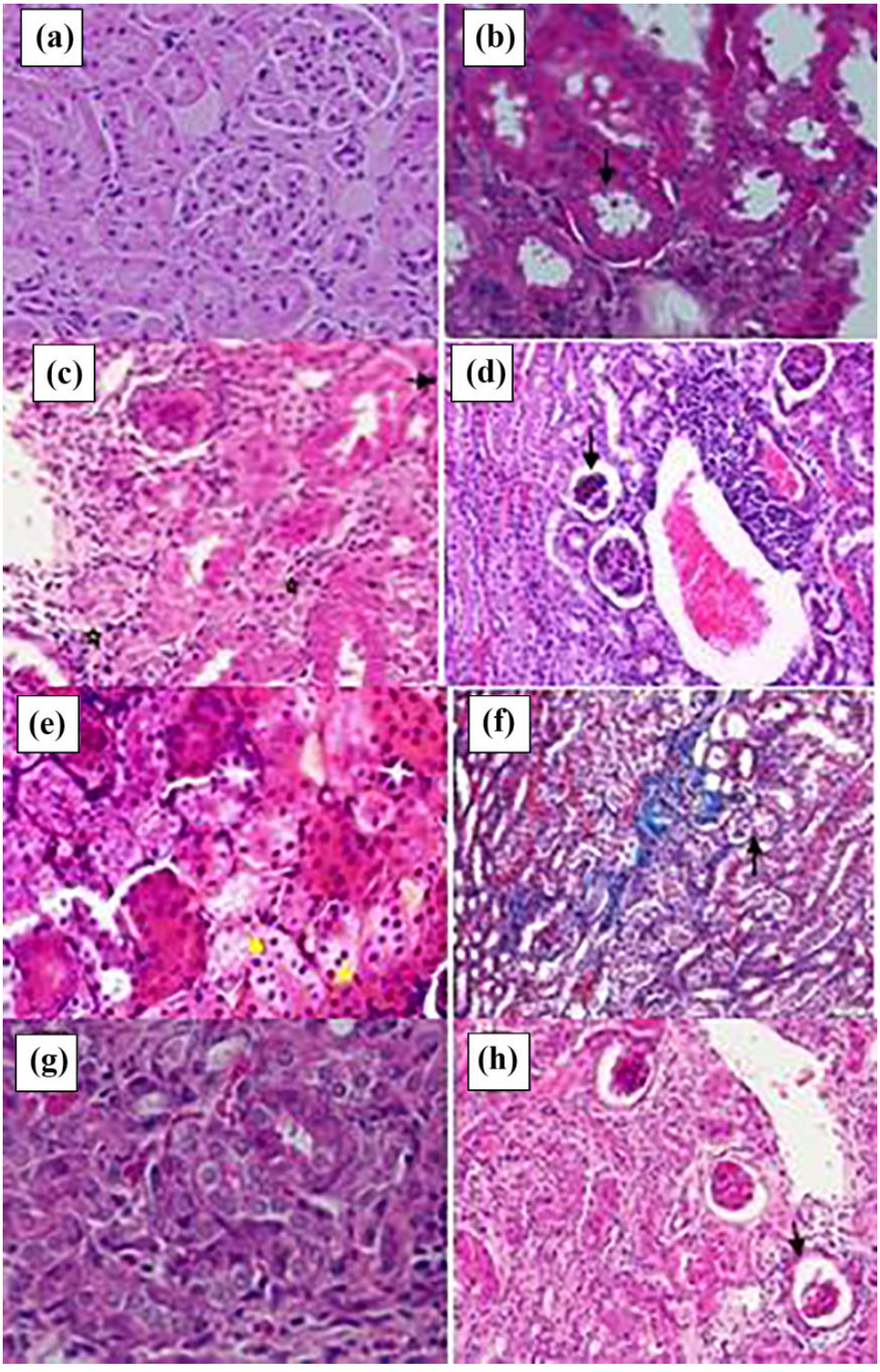

Figure 5. Renal histological alterations induced by different sizes of Ag NPs. (a) Normal kidney (270×). Note normal glomeruli together with normal proximal and distal renal tubules. (b) Tubular regeneration (arrow), 10 Ag NPs (270×). (c) Inflammatory cells infiltration (stars), 20 Ag NPs (270×). (d) Glomerular atrophy (arrows), 10 nm Ag NPs (135×). (e) Tubular basophilia (triangles), 10 Ag NPs (270×). (f) Renal tubules necrosis (arrow), 20 nm Ag NPs, Mallory trichrome stain (135×). (g) Fibrocytes proliferation, 10 nm Ag NPs (270×). (h) Glomerular shrinkage and Bowman's capsule thickening (arrow), 10 nm Ag NPs (135×). Ag NP: silver nanoparticle.

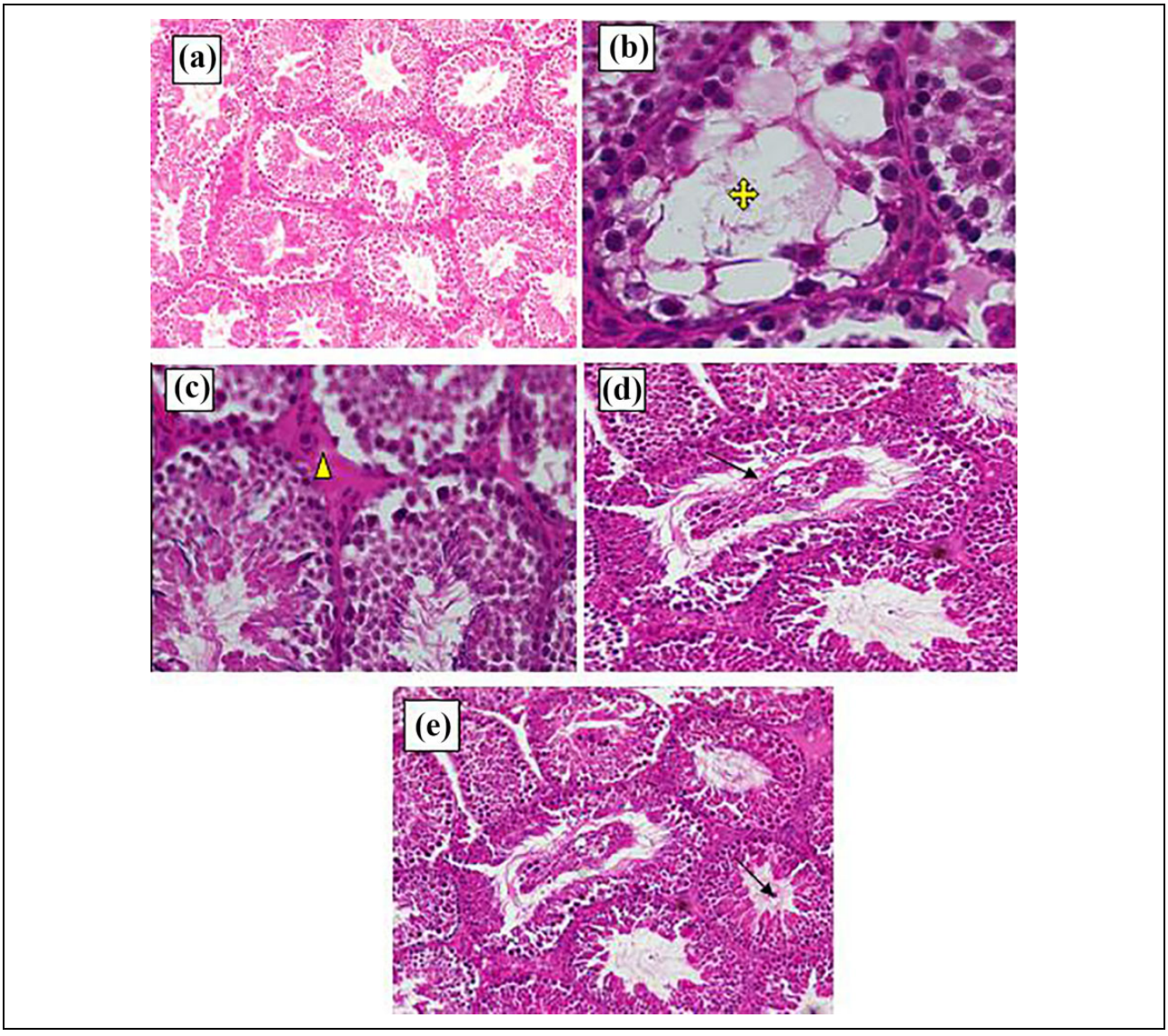

Figure 6. Testicular histological alterations induced by different sizes of Ag NPs: (a) normal seminiferous tubules with normal interstitial tissues (270×); (b) degenerative spermatogenic cells with cytoplasmic vacuolation (quad arrow), 10 nm Ag NPs (540×); (c) mild intertubular oedema (triangle), 10 nm Ag NPs (540×); (d) spermatocytes desquamation (arrow), 20 nm Ag NPs; and (e) spermatid giant cell (arrow), 20 nm Ag NPs (540×). Ag NP: silver nanoparticle.

(Figure 6(e)). This might have resulted from the effect of these NMs on the spermatocytes cytoskeleton, which may induce testicular damage and spermatogenesis abortion.

These current testicular histopathological findings as summarized in Table 3 are in line with reports from the Norwegian Institute of Public Health (Asare et al., 2012) that demonstrated testicular tissue injury due to Ag NPs exposure with possible consequences for male fertility.

The lack of facilities and insufficient funding were the main limitations to investigate the gene expression and ultrastructural alterations that might be induced by variable sizes of Ag NPs. Otherwise, the findings would be more informative, a subject to be considered for future work.

It may be concluded from the current work that Ag NMs could induce significant in-life alterations together with biochemical, hepatic, renal and testicular histological and histochemical changes that could affect the structure and functions of these vital organs. Moreover, it might be concluded that Ag NPs could induce injury in the hepatic tissues and to a lesser extent in the renal and the testicular tissues. In

addition, the results of the present study could suggest that nano-Ag materials could induce oxidative stress in the vital organs, with a clear conclusion that the smaller particles are more toxic than the larger ones.

Authors' note

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