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Pathological Evaluation of The Effect of Zinc Oxide Nanoparticles on Chromium-Induced Reproductive Toxicity in Male Albino Rats

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Abstract

The present study was intended to evaluate the effect of zinc oxide nanoparticles (ZnO-NPs) on chromium (Cr VI)-induced reproductive toxicity in male rats. Forty adult male albino rats were randomly divided into four equal groups (10 rats each), control group, chromium (Cr VI) group, potassium dichromate (K2Cr2O7) given orally at dose 10.5 mg/kg bwt/day (equals to 1/10 of LD50) (five days a week), Cr VI + ZnO-NPs group, rats received 10.5 mg/kg bwt/day (five days a week K2Cr2O7 orally and injected intraperitoneally (IP) by ZnO-NPs-saline solution at dose 5 mg/kg bwt/ day (three days a week). ZnO-NPs-group, ZnO-NPs- saline solution (IP) at dose 5 mg/kg bwt/ day (three days a week). Ten weeks post-treatment, serum testosterone values were significantly decreased in the Cr (VI) and Cr (VI) + ZnO-NPs-treated rats, sperm counts and significant increase in sperm abnormalities. No significant alteration in testicular malondialdehyde and superoxide dismutase levels among treated groups all over the experiment compared to control. However, reduced glutathione concentrations in testes were significantly decreased in Cr (VI) + ZnO-NPs and ZnO-NPs groups at ten weeks post-treatment. Cr (VI) group showed marked histopathological alterations and to lesser extent Cr (VI) + ZnO-NPs group that were time dependent. ZnO-NPs group showed significant testicular lesions, particularly after ten weeks post-treatment. It could be concluded that Cr (VI) intoxication induced damaging effects on male reproductive functions that were time dependent. ZnO-NPs partially improved these effects. But, its use for longer period has no valuable effect on Cr (VI)-induced toxicity.

1. INTRODUCTION

Chromium is an element naturally found in volcanic dust, earth crust and is widely distributed in the environment (Arreola-Mendoza et al., 2006). Naturally, trivalent Cr (III) and hexavalent Cr (VI) are most important chromium compounds. Hexavalent chromium is more toxic than trivalent chromium (Sugiyama, 1992). Moreover, Cr (VI) released from chromate industries as well as atmospheric emissions lead to environmental contamination with chromium (Banu et al., 2008). Cr (VI) has carcinogenic, genotoxic besides cytotoxic impacts in human and laboratory animals (Stohs et al., 2001). Cr (VI) compounds are easily transport across cellular plasma membrane through a non-selective anion channel that is normally utilized for uptake of physiologically relevant anions (Joiner et al., 1990). Reduction of Cr (VI) to Cr (III) inside the cell causes an over release of reactive oxygen species (ROS), which is mainly responsible for Cr (VI) toxicity (Stohs et al., 2001). Excessive generation of ROS can cause lipid peroxidation, DNA damage, apoptotic and necrotic cell death (Bagchi, et al., 2002). Several studies

reported the harmful effects of Cr (VI) on male reproductive functions in rats (Patel et al., 2014; Kumer and Siva, 2015; Al-Mukhtar et al., 2016). Nanoparticles are now used as transport systems for antineoplastic drugs, antibiotics, antifungal drugs and vaccines (Cashin-Garbutt and Hons 2012). Nano-materials have excessive potential to get access some cellular barriers to able reaching the cells and molecules in various diseases (Suri et al., 2007; Said et al., 2012). It is thought that zinc oxide nanoparticles (ZnO-NPs) are bio-safe and biocompatible and could be applied in biomedical materials (Berube, 2008). Recent studies reported the ameliorative effect of ZnO-NPs on male reproductive functions (Afifi et al., 2015; Hafez, 2015). Though, toxicological studies showed that ZnO-NPs had injurious effects on animals and human health. Since that the size of NPs surface area greatly augments their capability to produce ROS through oxidative stress (Moller et al., 2010). This study was designed to evaluate the outcome of using ZnO-NPs on chromium hexavalent toxicity in male rats on the bases of assessment of some reproductive functions, oxidative status and histopathology.

2. MATERIALS AND METHODS

Experimental animals

Seventy apparently healthy mature male Albino rats (170-180 g bwt) were obtained from Faculty of Agriculture, Alexandria University, Egypt. They were kept in clean metal cages in Pathology Department, Faculty of Veterinary Medicine with free access to get water and food. Rats were reserved two weeks before the experiment for adaptation with careful observation for any abnormalities.

Experimental design

Estimation of lethal dose 50 (LD50) of potassium dichromate (K2Cr2O7) in adult male albino rats

Twenty five adult male albino rats were distributed into five equal groups and orally given different doses of K2Cr2O7 (Cr VI) (50, 100, 150, 200, 250 mg/kg bwt). Another five animals were served as control. After one week close observation, mortalities were recorded in each group. LD50 were calculated according to Kerber formula that described by Turner (1965).

Assessment of protective effect of ZnO-NPs on Cr (VI)-induced reproductive toxicity in male albino rats

Forty adult male albino rats were randomly distributed into four equal groups (10 rats each), control group, Cr (VI) group, rats given potassium dichromate (K2Cr2O7) dissolved in distilled water orally at dose 10.5 mg/kg bwt/day (equals to 1/10 of LD50) (five days a week), Cr (VI) + ZnO-NPs group, rats received K2Cr2O7 dissolved in distilled water orally at dose 10.5 mg/kg bwt/day (equals to 1/10 of LD50) (five days a week) and injected intraperitoneally by ZnO-NPs-saline solution at dose 5 mg/kg bwt/ day (three days a week). ZnO-NPs-group, rats injected intraperitoneally by ZnO-NPs- saline solution at dose 5 mg/kg bwt/ day (three days a week). ZnO-NPs (nanoparticles <100 nanometer) were purchased from Sigma-Aldrich Company, Egypt. ZnO-NPs dose was selected according to Badkoobeh et al. (2013). The period of this study was 10 weeks.

Serum testosterone assay

At four and ten weeks, blood samples were obtained from each group shortly before euthanasia in clean tubes then centrifuged 10 minutes at 3000 rpm. Separated serum was preserved at -20 ^{o} C to estimate testosterone levels using radioimmunoassay kits (Bio Diagnostics Company, Cairo, Egypt).

Sperm characteristics

Cauda epididymis semen content was collected to estimate sperm count and sperm abnormalities. An eosin aqueous solution 2% was applied as a diluent then sperm counts were obtained using hemocytometer under a light microscope at ×200 magnification (Bearden and Fuquay, 1980). For detection of sperm abnormalities, percentages of morphologically abnormal sperms were documented among 300 counted sperms on each slide through microscopic examination at X400 magnification (Evans and Maxwell, 1987).

Relative organs weight

After four and ten weeks from the beginning of the experiment rats were euthanized and testes, epididymis and accessory sex glands were weighed. According to Matousek (1969), relative organs weight was calculated as follow I.W. =organ weight (g)/body weight (g) × 100.

Lipid peroxidation and antioxidant status

One testis of each rat was collected and homogenized in 9 volumes phosphate buffer then centrifuged at 3000 rpm for 15 minutes. Lipid peroxidation (LPO) was estimated as malondialdehyde (MDA). MDA, reduced glutathione (GSH) and superoxide dismutase (SOD) were measured spectrophotometrically according to Placer et al. (1966); Sedlak and Lindsay (1968) and Marklund and Marklund (1974), respectively using kits obtained from bio diagnostic Company Co. Egypt.

Histopathological examination

Following necropsy, tissue samples were taken from testes, epididymis, seminal vesicles and prostates. They rapidly fixed for at least 24 hours in 10% formalin solution then processed through routine paraffin embedding technique. Five micron-thick paraffin sections were stained by hematoxylin and eosin (HE) (Bancroft et al. (2013).

Statistical analysis

Statistical analyses were done by the Statistical Analysis System software (SAS, 2011). Effect of treatments on biochemical was assessed by the analysis of variance. Means were compared using Duncan's Multiple Range test at a significance level of $P \leq 0.05$. Values are presented as means and standard errors.

3. RESULTS

Serum testosterone analysis and sperm characteristics

Table 1 shows a significant decline in serum testosterone levels in the Cr (VI)-and Cr (VI) + ZnO-NPs-treated rats in comparison to control group that after 10 weeks post-treatment. But, the greatest decrease was observed in Cr (VI)-intoxicated rats. While, ZnO-NPs-treated rats showed non-significant decrease in testosterone levels compared to control rats. No significant variances were noted in sperm count and sperm abnormalities in all experimental groups during the first four weeks of the experiment compared to control (Table 1). However, ten weeks of treatment caused significant decrease in sperm count in all treated groups in relation to control one (Table 1).

Relative organs weight

As shown in table 1 at the first 4 weeks of the experiment, there were no significant changes in the testes and accessory sex glands weight in all experimental groups in relation to control. While the relative epididymis weight presented a significant rise in Cr (VI) intoxicated rats and no significant alteration in Cr (VI) + ZnO-NPs and ZnO-NPs groups as compared to control one. After 10 week of the experiment, testes relative weight showed a significant increase in Cr (VI) + ZnO-NPs treated rats and no significant differences in other groups compared to control group. While the epididymis relative weight showed a significant decrease in Cr (VI) + ZnO-NPs and ZnO-NPs treated rats, while no significant change between chromium and control group. Also, the accessory sex glands relative weight displayed a significant increase in Cr (VI) + ZnO-NPs group and non-significant changes in other groups as compared to control group (Table 1).

Testicular MDA, GSH and SOD assays

Table 2 displays no significant difference in testicular MDA and SOD levels among all experimental groups at the two time points of the study compared to control group. Concerning testicular GSH levels, a significant reduction was noticed in Cr (VI) + ZnO-NPs and ZnO-NPs groups at 10 weeks of the experiment compared to control.

Histopathologic results

Testes

Control rats had normal testicular histologic structure with rounded and uniformly arranged seminiferous tubules that had multiple layers of spermatogenesis ended by sperm (Fig. 1a). After 4 weeks of the experiment, Cr (VI) - intoxicated rats showed atrophied disorganized seminiferous tubules, incomplete spermatogenesis and bucked irregular basement membrane. Moreover, many seminiferous tubules had necrotic sloughed germinal epithelium (Fig. 1b). There was moderate interstitial edema represented by faint eosinophilic material and congestion of intertubular blood vessels. The testes of rats treated with Cr (VI) + ZnO-NPs showed mild interstitial edema and slight vacuolation of germinal epithelium. Few numbers of seminiferous tubules were atrophied with single layer of germinal epithelium, and irregular basement membrane (Fig. 1c). No histopathological lesions were detected in the testes of rats treated by ZnO-NPs (Fig. 1d). After ten weeks of the experiment, Cr (VI) rats exhibited similar lesions observed at four weeks of the experiment but with increased severity and distribution. Most seminiferous tubules were free from spermatozoa (Fig. 1e), other tubules contained increased number of sloughed germinal epithelium and degenerated spermatids. Furthermore, focal aggregation of multinucleated giant cells was detected in many seminiferous tubules (Fig. 1f) with severe intertubular edema and congestion. Cr (VI) + ZnO-NPs treated rats had mild thickening, hyalinization and buckled basement membrane of seminiferous tubules with vacuolated germinal epithelial cells, mild vascular congestion and interstitial edema (Fig. 1g). In addition, lumina of seminiferous tubules contained moderate number of degenerated spermatid and sloughed germinal epithelium. Testes of ZnO-NPs treated rats showed atrophied tubules without sperms. Other seminiferous

tubules showed vacuolar degeneration of spermatogonia and Sertoli cells. Moreover, there was obvious incomplete spermatogenesis in some seminiferous tubules. Furthermore, multinucleated giant cells were noticed with mild interstitial edema and congestion (Fig. 1h).

Epididymis

Epididymis of control rats showed normal histologic structure with considerable sperm density all over the experimental period (Fig. 2a). At four weeks of the experiment, Cr (VI)-treated rats had some epididymal ductules with slight vacuolation of germinal epithelium and low density of spermatozoa (Fig. 2b). In addition to mild to moderate interstitial edema with mononuclear cells infiltration and intertubular capillary congestion. Few epididymal ductules of Cr (VI) + ZnO-NPs treated rats were free of sperms (Fig. 2c) and appeared histologically normal. As well, the epididymal ductules of ZnO-NPs treated rats were impacted with sperms (Fig. 2d) with normal histologic structure. At ten weeks of the experiment, Cr (VI) treated rats showed hyperplasia of the lining epithelial cells of epididymal ductules, other tubules had sloughed germinal epithelium, interstitial edema, and mild mononuclear cells infiltration. Some epididymal ductules had low number of spermatozoa (Fig. 2e). ZnO-NPs treated rats had few number of epididymal ductules with low density of spermatozoa (Fig. 2f).

Accessory sex glands

Seminal vesicles (Fig. 3a) and prostate gland of control rats appeared with a normal histological boundary all over the experimental period. After four weeks, all experimental groups showed normal histologic structure with considerable amount of eosinophilic secretions. After ten weeks of the experiment, seminal vesicles of Cr (VI) treated rats showed congestion and inflammatory cells infiltration surrounded blood vessels (Fig. 3b). Prostate gland of Cr (VI) + ZnO-NPs treated rats exhibited mild necrosis and desquamation of some tubuloalveolar glandular epithelial, mild interstitial edema and perivascular infiltration of lymphocytes (Fig. 3c). The seminal vesicles and prostate glands of ZnO-NPs treated rats were normal in architecture (Fig. 3d).

Table 1 Effect of Cr (VI) and ZnO-NPs administration on serum testosterone (ng/ml) levels, sperm charachteristics and relative reproductive organs weights.

Parameters	Time (week)	Groups			
		Control	Cr (VI)	Cr (VI)+ZnO-NPs	ZnO-NPs
Serum testosterone (ng/ml)	10	2.82±0.33^{a}	0.95±0.52^{b}	1.59±0.20^{b}	1.81±0.21^{ab}
Sperm count (10^6/ml)	4	65.73±12.14^{a}	66.87±18.27^{a}	73.85±14.20^{a}	42.00±3.46^{a}
	10	135.30±6.51^{a}	47.98±15.81^{b}	77.53±18.17^{b}	86.00±27.83^{b}
Sperm abnormalities (%)	4	84.49±2.74^{a}	88.32±3.14^{a}	88.08±3.34^{a}	93.23±1.94^{a}
	10	83.27±4.26^{a}	88.43±4.53^{a}	87.46±0.76^{a}	81.53±5.79^{a}
Testes I.W.	4	0.92±0.08^{a}	1.13±0.05^{a}	1.07±0.05^{a}	0.90±0.09^{a}
	10	1.07±0.02^{b}	1.08±0.10^{b}	1.65±0.31^{a}	1.02±0.15^{b}
Epididymis I.W.	4	0.51±0.03^{b}	0.76±0.08^{a}	0.55±0.03^{b}	0.53±0.05^{b}
	10	1.27±0.13^{a}	1.22±0.07^{a}	0.71±0.06^{b}	0.85±0.13^{b}
accessory sex gland I.W.	4	0.65±0.07^{a}	0.63±0.06^{a}	0.65±0.04^{a}	0.82±0.08^{a}
	10	0.56±0.08^{b}	0.79±0.15^{ab}	0.94±0.09^{a}	0.66±0.04^{b}

All values are expressed as mean±S.E. Values with different letters at the same row are significantly different at$P\leq0.05$ (ANOVA) with Duncan's multiple range test. Index Weight (I.W.) = organ weight (g)/ body weight (g) × 100.

Table 2 Effect of Cr (VI) and ZnO-NPs administration on testicular malondialdehyde (MDA), reduced glutathione (GSH) and superoxide dismutase (SOD) levels.

Parameters	Time (week)	Groups			
		Control	Cr (VI)	Cr (VI)+ZnO-NPs	ZnO-NPs
MDA (nmol /g)	4	10.67±1.76^{a}	0.19±0.01^{a}	0.20±0.01^{a}	13.67±2.03^{a}
	10	19.33±0.67^{a}	20.67±0.33^{a}	22.00±1.53^{a}	21.00±1.53^{a}
GSH (μmol/g)	4	0.29±0.03^{a}	0.19±0.01^{a}	0.20±0.01^{a}	0.18±0.04^{a}
	10	0.72±0.10^{a}	0.66±0.08^{a}	0.40±0.07^{b}	0.45±0.03^{b}
SOD (IU/mg)	4	243.67±4.33^{a}	279.67±26.36^{a}	258.67±28.67^{a}	302.33±11.29^{a}
	10	220.67±17.36^{a}	185.33±13.38^{a}	216.67±37.12^{a}	184.67±5.36^{a}

All values are expressed as mean±S.E. Values with different letters at the same row are significantly different at$P\leq0.05$ (ANOVA) with Duncan's multiple range test

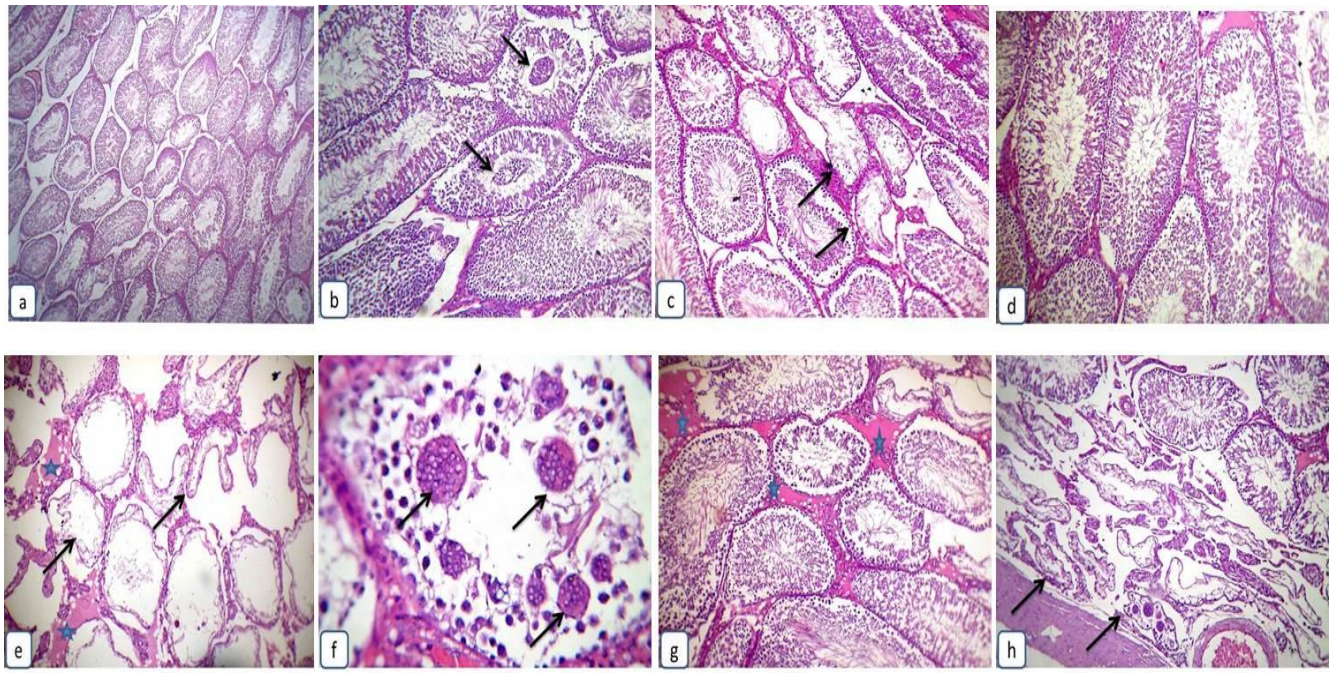

Figure 1 Testes of a rat stained by HE. (a) Control rat. (X 100). Four weeks of the experiment, (b) Cr (VI)-treated group, Sloughed germinal epithelial cells (arrows) (X 250). (c) Cr (VI) + ZnO-NPs- treated group, Small seminiferous tubules with single layer of germinal epithelium and wavy basement membrane (arrows) (X 250). (d) ZnO-NPs-treated group, Normal seminiferous tubules with complete spermatogenesis (X 250). Ten weeks of the experiment. Cr (VI)-treated group. (e) Most seminiferous tubules were free from spermatozoa (arrows) (X 250), (f) Aggregation of multinucleated giant cells (arrows) (X 400). (g) Cr (VI) + ZnO-NPs-treated group, Mild interstitial edema (asterisks) (X 250). (h) ZnO-NPs-group, Degenerated buckled seminiferous tubules (arrows) with multinucleated giant cells were noticed with mild interstitial edema and congestion (X 250).

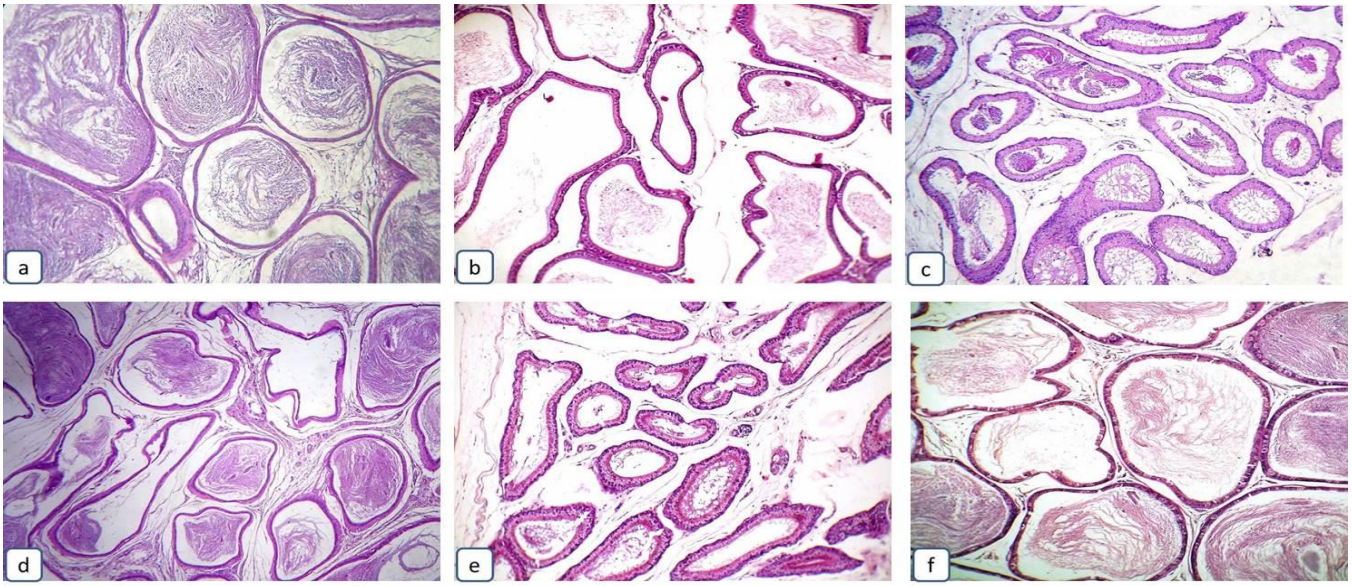

Figure 2 Epididymis of a rat stained by HE. (X 100). (a) Control rat showed normal histological structure with normal sperm density. Four weeks of the experiment. (b) Cr (VI)-treated group, Some epididymal ductules had low density of spermatozoa. (c) Cr (VI) + ZnO-NPs- treated group, Few epididymal ductules were free of sperms. (d) ZnO-NPs-treated group, Epididymal ductules were impacted with sperms. Ten weeks of the experiment. Cr (VI)-treated group. (e) Some epididymal ductules had minimal density of spermatozoa. (f) ZnO-NPs-treated group, Few number of epididymal ductules with low density of spermatozoa.

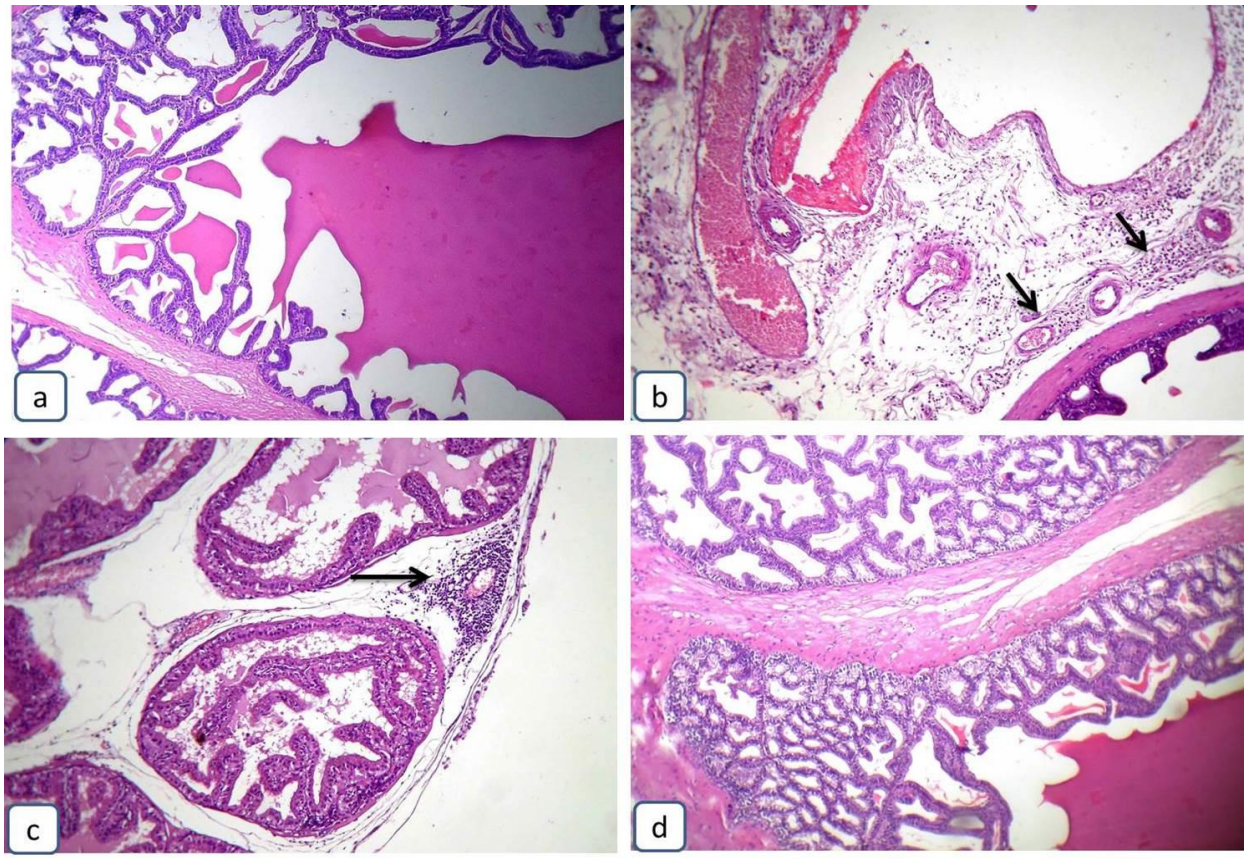

Figure 3 (a) Seminal vesicle of a control rat with normal histological structure. HE. (X 100). (b) Seminal vesicle of Cr (VI) treated rat, Congestion and perivascular lymphocytic infiltrations (arrows). HE (X 100) (c) Prostate gland of Cr (VI) + ZnO-NPs treated rat, Perivascular lymphocytic infiltration (arrow). HE. (X 100). (d) Seminal vesicle of ZnO-NPs-treated rat, normal histologic structure. HE. (X 100).

4. DISCUSSION

Testis is considered as one of the main target organ for metal-induced oxidative damage as a result of its rich content of polyunsaturated membrane lipids (Acharya et al., 2004). Based on pathological results of our investigation, it was clear that the toxic potential of Cr (VI) was predominantly time-dependent. Moreover, the majority of the recorded lesions in various organs within a given treatment regimen were much more in severity and distribution during ten weeks than those noticed after four weeks of the experiment. Administration of Cr (VI) induced pathological results on reproductive system of male rats and this could be attributed to chromium induced oxidative damage (Stohs et al., 2001). Our pathological results were comparable to those reported by Chandra et al., (2007) and Rankov et al., (2010). Moreover, the recorded significant reduction in serum testosterone level of Cr (VI) intoxicated rats after ten weeks has been reported by Marouani, (2011); Jeber and Tawfeek, (2013); Al-Mukhtar et al. (2016). This variation could be contributed to the intracellular reduction of Cr (VI) producing pentavalent (Cr V) form of chromium that may disrupt the blood-testis barrier and affect the functions of Sertoli cells (Pereira et al., 2002). Low level of serum testosterone in Cr (VI) treated rats may correlate to the spermatogenic impairment and depleted epididymal sperm number (Chandra et al., 2007) as, testosterone is essential for the motivation and conservation of spermatogenesis (Singh et al., 1995). In the same way, the Cr (VI) treated rats showed significant drop in sperm count with numerical increase in sperm abnormalities after ten weeks (Yousef et al., 2006; Akunna et al., 2012). Such decrease may be due to disturbance in steroidogenesis as well as alterations in serum gonadotropin and testosterone levels (Saxena et al., 1990). On the other hand, treatment of ZnO-NPs did not restore the decrease in testosterone level and the change in sperm count to normal (Shirvani, 2014). In

contrast, Badkoobeh et al., (2013) reported that ZnO-NPs treatment induced non- significant decrease in serum testosterone level. This variation may be due to the difference in administration period. Moreover, administration of ZnO-NPs in concomitant with chromium resulted in retrieving the histopathologic alterations in testes and epididymis after one month of experiment. While, it failed to restore the chromium-induced testicular and epididymal damages after 10 weeks. Where, ZnO-NPs treated rats showed obvious incomplete spermatogenesis in the most of seminiferous tubules. Furthermore, multinucleated giant cells were noticed, with mild interstitial edema and congestion. Regarding epididymis, few number of epididymal ductules was devoid of spermatozoa. These results agree with Shirvani, (2014) and El-Morshedi, (2014). It is noteworthy that nanoparticles can generate reactive oxygen (ROS) and membrane lipid peroxidation and finally lead to apoptosis and cell death (Lee et al., 2012; Dadong et al., 2013). Concerning to MDA, GSH and SOD did not vary significantly after four weeks, while after 10 weeks there was a significant drop of testicular GSH levels in Cr (VI) + ZnO-NPs and ZnO-NPs treated rats and an increase in MDA values numerically at all groups compared to control rats. MDA is used as a biomarker of oxidative stress. MDA may damage proteins and lipids present in cell membrane (Bagchi, et al., 1995). The free radical production that induced by chromium may lead to exhaustion of the antioxidants enzymes due to their use in the scavenging free radical (Wang et al., 2006 and Shati 2013). The relative testes weight showed significant rise in Cr (VI) + ZnO-NPs- treated rats after ten weeks that may be result from edema and congestion. While, epididymis relative weight showed significant decrease at Cr (VI) + ZnO-NPs and ZnO-NPs rats which might occur due to decrease sperms count. Our investigation revealed that Cr (VI) rats showed normal structure of seminal vesicle and prostate gland after one month of duration. While after ten weeks Cr (VI) rats showed seminal vesiculitis which represented by congestion and inflammatory cells infiltration. Also prostate gland had necrotic and desquamated glandular epithelial cells debris, mild interstitial edema and perivascular infiltration of lymphocytes. The prostatic lesions were parallel to Rankov et al., (2010). These histopathological findings were due to oxidative damage of such tissues as a result of Cr (VI) intoxication. On other hand, Cr (VI) + ZnO-NPs treated rats showed normal histological limits of seminal vesicles and prostate gland after one month of experiment. ZnO-NPs did not disturb significantly LPO and antioxidant activity, particularly at four weeks of the experiment (Sharma et al., 2012), but it decreased GSH at the end of the experiment. It could be concluded that Cr (VI) intoxication induced deleterious effects on male reproductive functions and these effects were time dependent. The use of ZnO-NPs partially improved the toxic effect of Cr (VI) during the first time point of the experiment. But, its use for longer period has no valuable effect on Cr (VI)-induced toxicity.

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