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Original Article

Protective effects of ascorbic acid and zinc against cadmium-induced histopathological, histochemical and cytogenetic changes in rats

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Abstract

The present study aimed to investigate the protective role of ascorbic acid (vitamin C) and zinc (Zn) against cadmium (Cd) induced histopathological changes in tissues of liver, kidney, lung and testis of rats as well as chromosomal aberrations of bone marrow. For this purpose, 60 male albino rats were divided into six groups; each group contained 10 animals. The first group served as control and was given only distilled water. The second and third groups received 200 mg Vit. C/kg b.w. and 50 mg Zn/kg b.w., respectively. The fourth group received daily oral dose containing 3 mg Cd/kg b.w. (1/30 LD_{50}). The fifth group received Cd+Vit. C (3 mg Cd/kg b.w. + 200 mg Vit. C/kg b.w.), while the sixth group received Cd+Zn (3 mg Cd/kg b.w. + 50 mg Zn/kg b.w.). The treatment in all groups was lasted for 90 consecutive days. The results indicated that rats exposed to cadmium showed severe histopathological changes in liver, kidney, lung and testicular tissues as well as significantly increased in the frequency of chromosomal aberrations such as: break, ring, centromeric separation and polyploidy. Co-treatment with zinc partially improved the histopathological changes and decreased the frequency of chromosomal aberrations while co-treatment with vitamin C exhibited more protective role and markedly reduced tissues damage induced by Cd.

Keywords: cadmium, ascorbic acid, zinc, histopathology, chromosome aberrations

Efeitos protetores de ácido ascórbico e zinco contra alterações histopatológicas, histoquímicas e citogenéticas induzidas por cádmio em ratos

Resumo

O presente estudo teve como objetivo investigar o papel protetor do ácido ascórbico (vitamina C) e zinco (Zn) contra as alterações histopatológicas induzidas por cádmio (Cd) em tecidos do fígado, pulmão, rim e testículo de ratos, bem como aberrações cromossômicas de medula óssea. Para este fim, 60 ratos albinos machos foram divididos em seis grupos, cada grupo continha 10 animais. O primeiro grupo serviu como controle e recebeu apenas água destilada. Os segundo e terceiro grupos receberam 200 mg Vit. C/kg de peso corporal e 50 mg de Zn/kg de peso corporal, respectivamente. O quarto grupo recebeu uma dose oral diária que contém 3 mg de Cd/kg de peso corporal (1/30 LD50). O quinto grupo recebeu Cd + Vit. C (3 mg Cd/kg pc + 200 mg Vit. C/kg de peso corporal), enquanto que o sexto grupo recebeu Cd + Zn (3 mg Cd/kg pc + 50 mg de Zn/kg de peso corporal). O tratamento em todos os grupos durou 90 dias consecutivos. Os resultados indicaram que os ratos expostos ao cádmio mostraram graves alterações histopatológicas em tecidos do fígado, pulmão, rim e dos testículos, bem como aumento significativo na frequência de aberrações cromossômicas, tais como: quebra, anel, separação centromérica e poliploidia. Cotratamento com zinco melhorou parcialmente as alterações histopatológicas e diminuiu a frequência de aberrações cromossômicas, enquanto o cotratamento com vitamina C apresentou papel mais protetor e marcadamente reduzido número de tecidos com danos induzidos por Cd.

Palavras-chave: cádmio, ácido ascórbico, zinco, histopatologia, aberrações cromossômicas

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Introduction

Cadmium (Cd) is known to be one of the most toxic environmental and industrial pollutants. Its industrial applications were developed based on its unique chemical and physical properties (Krichah et al., 2003). Cd is one of the most harmful heavy metals and it is able to induce severe injury (Suzuki et al., 1989). People who live near hazardous waste sites or factories that release cadmium into the air and people that work in metal refinery industry have been shown to suffer from impaired health, such as damaged lungs, diarrhea, stomach pains and severe vomiting, bone fracture, reproductive failure and possibly even infertility (Nordberg, 2009), damage on central nervous system, psychological disorder, possibly DNA damage or cancer development (Singh et al., 2007; Amara et al., 2011). It accumulates and proves to cause severe damage to a variety of organs such as lung, brain, testis, kidney, liver, blood system and bone (Ercal et al., 2001; Ersan et al., 2008; Cucu et al., 2011).

Cadmium is known to deplete glutathione and protein-bound sulfhydryl groups, which results in enhanced production of reactive oxygen species (ROS), such as superoxide ion, hydroxyl radicals and hydrogen peroxide (Liu et al., 2001). As mentioned, cadmium exerts its toxic effects via oxidative damage to cellular organelles by inducing the generation of (ROS); reactions of these ROS with cellular biomolecules have been shown to lead to lipid peroxidation, membrane protein damage, altered anti-oxidant system, DNA damage, altered gene expression and apoptosis (Stohs et al., 2000; Wu et al., 2002). If these ROS-mediated stress events are not balanced by repair processes, they can affect cells to undergo apoptosis or necrosis (Thevenod, 2003). As oxidative stress is one of the important mechanisms of cadmium-induced damages, it can be expected that thw administration of some antioxidants should be an important therapeutic approach (Sinha et al., 2009; Renugadevi & Prabu, 2010).

Ascorbic acid (Vit. C) is a water-soluble dietary antioxidant that plays an important role in controlling the oxidative stress (Panayiotidis & Collins, 1997; Kini et al., 2011). It has been reported that ascorbic acid enhances cadmium transport and decreases its uptake in rat intestinal segments (Tarasub et al., 2012). It has also been demonstrated that vitamin C is one of the most effective factors reducing an enhanced renal and hepatic cadmium burden in pigs fed on a diet enriched with copper (Kapl et al., 1994).

Zinc (Zn) is the most abundant trace intracellular element required for a number of cellular processes, including cell proliferation, reproduction, immune function and defense against free radicals (Powell, 2000). Indeed, increasing evidence suggests that zinc plays an important role as an antioxidant and protects cellular components from oxidation. Zn is one of the most important nutritional factors influencing the metabolism and toxicity of heavy metals, including Cd. As well as, Bruno et al. (2007) have indicated that zinc deficiency either *in vitro* or *in vivo* increased free radical production or increased oxidative damage occurs.

Consequently, this study was performed to elucidate the protective role of Vit C and Zn on genotoxic and histopathological effects induced by Cd on the liver, kidney, lung and testis of male albino rats.

Material and Methods

Chemicals

Cadmium chloride was obtained from Merck (Darmstadt, Germany). Zinc chloride and L-ascorbic acid were purchased from Sigma (St. Louis, MO, USA). All other chemicals were of analytical grade and were purchased from standard commercial suppliers.

Tested animals

Adult male albino rats (Sprague-Dawely), *Rattus norvegicus* var. albinus, weighing 180 ± 10g were purchased from the Biological Products and Vaccines Holding Company, Helwan Farm, Cairo, Egypt. Rats were kept under the laboratory conditions of 25 ± 5°C and 65 ± 5% relative humidity, with a 12 h light/dark cycle, for two weeks as an adaptation period. They were housed in stainless steel cages (35 x 25 x 20 cm) and maintained on *ad libitum* diet and water. The background levels of cadmium, Vit. C and Zn in the diet were 0.06, 0.00, and 8.40 mg/kg, respectively, according to the manufacturer's information. All treatments and procedures were in accordance with the protocol of National's Animal Care and Use Committee and Guidelines for the Care and Use of Experimental Animals.

Assessment of the oral LD_{50}

Calculating the median lethal dose (LD_{50}) for cadmium on male albino rats was performed in accordance with the Organization of Economic Cooperation and Development guidelines (OECD, 2001). Twenty five adult male rats with a body weight 180 ± 10g were randomly divided into five groups (n=5). Cadmium was dissolved in distilled water, and administered orally at doses of 40, 60, 90, and 135 mg/kg b. wt to single male rats, while control group received distilled water only. All the tested rats were observed shortly after dosing, then each rat was observed daily for a period of 14 consecutive days and mortality counts were recorded. The LD_{50} values were calculated according to the statistical method of Weill (1952).

Experimental design

Rats were randomly divided into six groups (10 rats/ group). The first group served as control and was given only distilled water. The second

and third groups received 200 mg Vit. C/kg b.w. and 50 mg Zn/kg b.w., respectively. The fourth group received a daily oral dose containing 3 mg Cd/kg b.w (1/30 LD_{50}). The fifth group received Cd+Vit C (3 mg Cd/kg b.w. + 200 mg Vit. C/kg b.w.) and the sixth group received Cd+Zn (3 mg Cd/kg b.w. + 50 mg Zn/kg b.w.).

Water consumption was determined daily to calculate the average administered dose of Vit. C and zinc per day (result not shown). We chose to administer the three elements by oral route because it is the main mode of exposure to Cd in humans and animals (Herranz et al., 2010). Cadmium, Vit C and Zn doses were chosen on the basis of available literature data (Grosicki, 2004; Jihen et al., 2009). The treatment in all groups was lasted for 90 consecutive days.

Histological and histochemical studies

At the end of experiment, liver, kidney, lung and testis from each sacrificed rat were dissected out; trimmed of excess fat. All tissues were fixed in 10% buffered formalin and were processed for paraffin sectioning by dehydration in different concentrations of alcohol, cleared with xylol and embedded in paraffin blocks. Sections of about 5µm thickness were stained with Harris haematoxylin and eosin (H&E) for histological study (Delafield, 1984), azan stain to demonstrate the collagenous fibers (Humason, 1972) and Feulgen's reaction to demonstrate DNA (Bancroft, 1996).

Cytogenetical study

From each experimental group, five rats were intraperitoneal injected with colchicine 2-3 h before sacrificing. The femurs bones were removed and bone-marrow metaphases were prepared according to the method of Yosida & Amano, (1965). Mitotic chromosome preparations were made through the air-drying technique of Ford & Hamerton (1956). The bone marrow of both femurs was put in 0.075 m KCl for 40 min at 37 ºC and fixed twice in a mixture of methanol: acetic acid (3:1). Chromosome slides were prepared by dropping the cell suspension onto ethanol cleaned slides and flaming them slightly. After air-drying, slides were stained with 7% Giemsa in phosphate buffer (pH 6.8). About 100 well spread metaphases per animal were analyzed for chromosome aberrations.

Statistical analysis

All values were expressed as mean ± SE for five animals (n = 5) in each group. Significant differences between the groups were determined with SPSS 10.0 software (SPSS Inc., Chicago, IL, USA) by performing one-way ANOVA with post hoc comparisons followed by Duncan's multiple range test. The results of cytogenetic study were statistically analyzed using t- test. A difference was considered significant at the $p < 0.05$ level.

Results

Results demonstrated that the oral LD_{50} value of cadmium to male albino rats was 90 mg/kg body weight.

Histological study

Haematoxylin and Eosin stain

The hepatic parenchyma of the control rats consisted of several hepatic lobules separated from each other by very delicate connective tissue septa housing the portal triad. Each hepatic lobule contained a thin walled central vein surrounded by hepatic cords radiating towards the periphery. *The portal area is including a hepatic portal vein, a branch of hepatic artery and a bile ductile* (Figures1a&b). Treatment with Cd caused severe liver damage including fatty changes, focal necrosis, pyknotic nuclei, karyolysis, proliferation of kupffer cells and bile ductless (Figures 1c,1d,1f). These histopathological changes were improved in the liver of rats treated with Cd plus Vit.C; the hepatocyte appeared with normal pattern while proliferation of bile ductless and dilatation of portal vein were still found (Figure 1g). The liver of rat treated with Cd plus Zn showed partially improvement of hepatocyte, few lipid droplets and little focal necrotic area (Figure 1h).

The kidney of the control rats showed the normal histological structure of the renal corpuscles and renal tubules. The renal corpuscle consisted of tuft of blood capillaries surrounded by the Bowman's capsule. The renal tubules included proximal convoluted tubules lined by large pyramidal cells with brush border and distal convoluted tubules lined by cuboidal cells (Figure 2a).

The rats treated with Cd (group 4) exhibited histopathological changes; shrunken or degeneration of glomerular tuft, cytoplasmic degeneration in cells of renal tubules, pyknotic nuclei, some tubules are necrotic, multiple foci of haemorrhage, dilatation and congestion of blood vessels (Figures 2b, 2c, 2d, 2e). These pathological lesions induced by Cd were remarkably reduced by administration of Cd plus Vit.C, since the glomeruli and renal tubules appeared as approximately similar to control (Figure 2f); while the rats treated with Cd plus Zn exhibited partially improvement of glomeruli and the renal tubules. Some pyknotic nuclei were observed in the cells of renal tubules (Figure 2h).

Lung sections of rats in the control group have shown normal appearance of alveoli, alveolar space and bronchioles (Figures 3a, 3b). In rats group treated with 3 mg/kg bw of Cd for 90 days, the lung sections have shown signs of histopathological changes, such as; oedma, thickening of interalveolar septa, enlargement of some air space, aggregation of lymphocyte infiltration, dilatation and congestion of pulmonary vein (Figures 3c, 3d, 3e, 3f). Rats treated with Cd plus Vit.C and Cd plus Zn exhibited no inflammatory cells, obvious improvements of bronchiole; however, some air space still enlarged (Figure 3g, 3h), respectively.

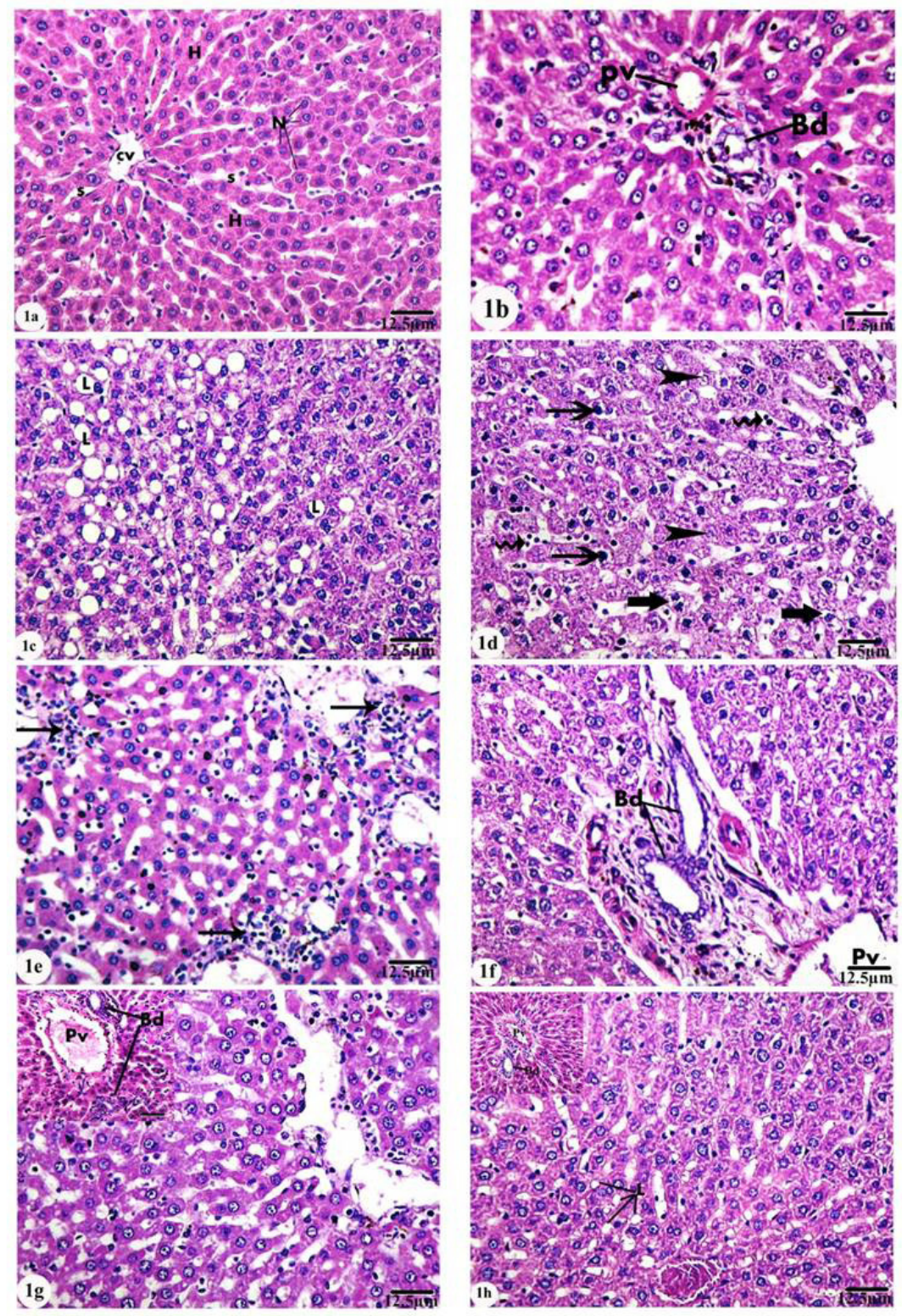

Figure 1. Sections in the liver stained with H&E showing: (a&b); hepatic tissue of control rat group showing normal hepatic architecture, hepatocyte (H), with their normal nuclei (N), sinusoids (s) and central vein (cv). Also, portal tract with bile ductless (Bd) and portal vein (pv) is observed. Hepatic tissues of Cd treated group showing (c); loss of hepatic architecture and many fatty droplets (L), (d); vacuolated cytoplasm (thick arrows), pyknotic nuclei (thin arrows), karyolysis (head arrows), proliferation of kupffer cells (irregular arrows), (e); focal necrotic areas accompanied with inflammatory cell infiltrations (arrows). (F); proliferation of bile ductless (Bd), (g); hepatic tissue of rat treated with (Cd+Vit.C) showing normal hepatocyte, dilatation of portal vein (pv) and proliferation of bile ductless (Bd) and (H); Hepatic tissue of rat treated with (Cd+Zn) showing partially improvement of hepatocyte, few lipid droplets (L), hyalinization area around portal vein (pv) and proliferation of bile ductless (Bd). Original magnification of each figure; x 200

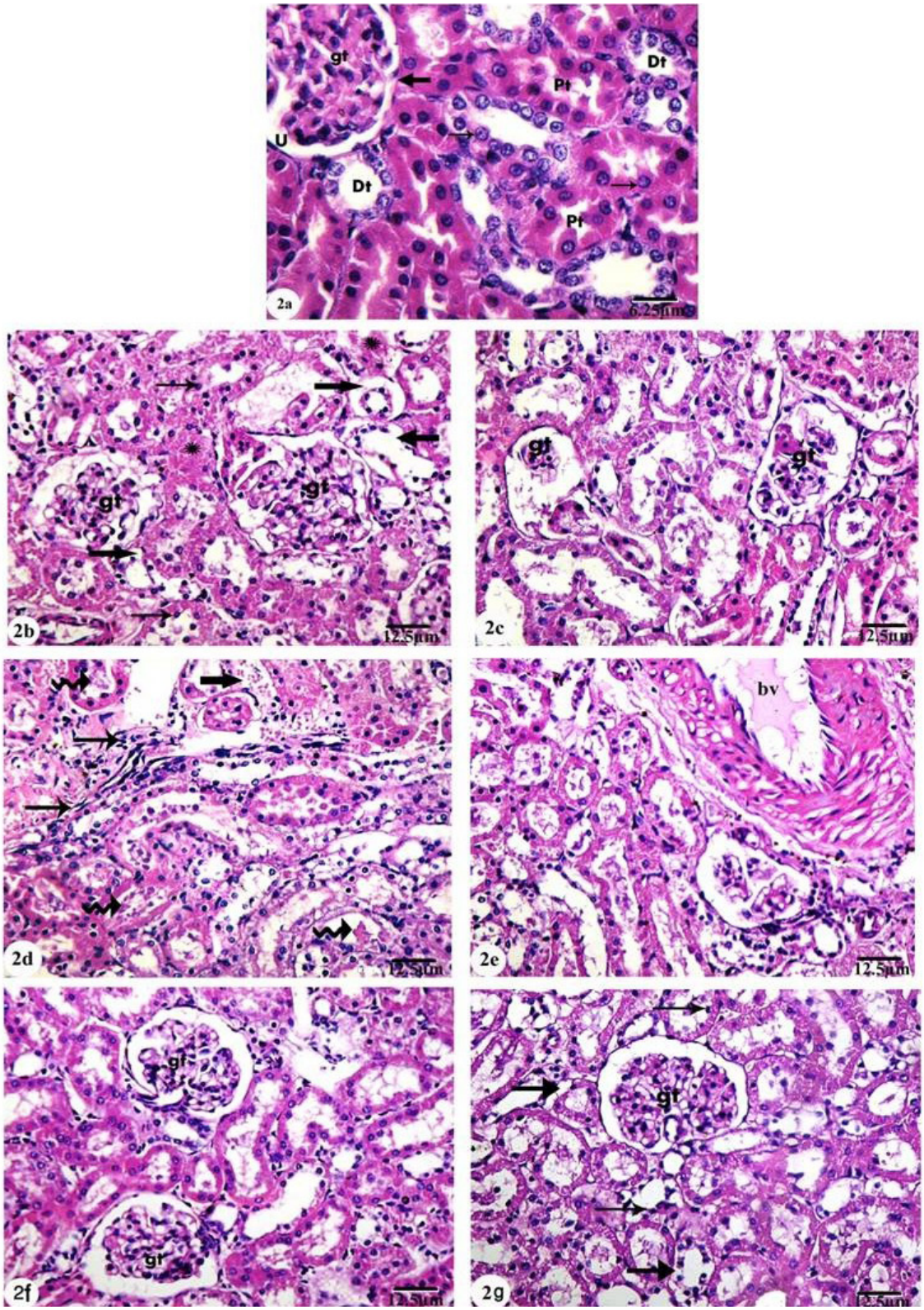

Figure 2. Photomicrographs of sections in the cortex of kidney stained with H&E showing (a); renal tissue of control group demonstrating normal appearance of glomerular tuft (gt), urinary space (U), Bowman's capsule (thick arrow), proximal tubule (pt), distal tubules (Dt) with their nuclei (thin arrows). Renal tissue of Cd treated rat showing (b); disrupted of Bowman's capsule, degenerated cytoplasm of some cells of renal tubules (thick arrows), some tubules are necrotic (*), pyknotic nuclei (thin arrows), (c); shrunken and degeneration of glomeruli, (d); multiple foci of hemorrhage (thick arrow), deposited material in renal tubules (irregular arrows), inflammatory cell infiltrations (thin arrows), (e); dilatation and congestion of blood vessels (bv), (f); renal tissue of rat treated with Cd+Vit.C showing improvement in glomerular tuft and renal tubules and (g); renal tissue of rat treated with Cd+Zn showing improvement of glomerular tuft, degenerated cytoplasm of some cells of renal tubules (thick arrows) and some pyknotic nuclei (thin arrows). Original magnification of (2a); x 400, Original magnification of (2b, c, d, e, f and g); x 200

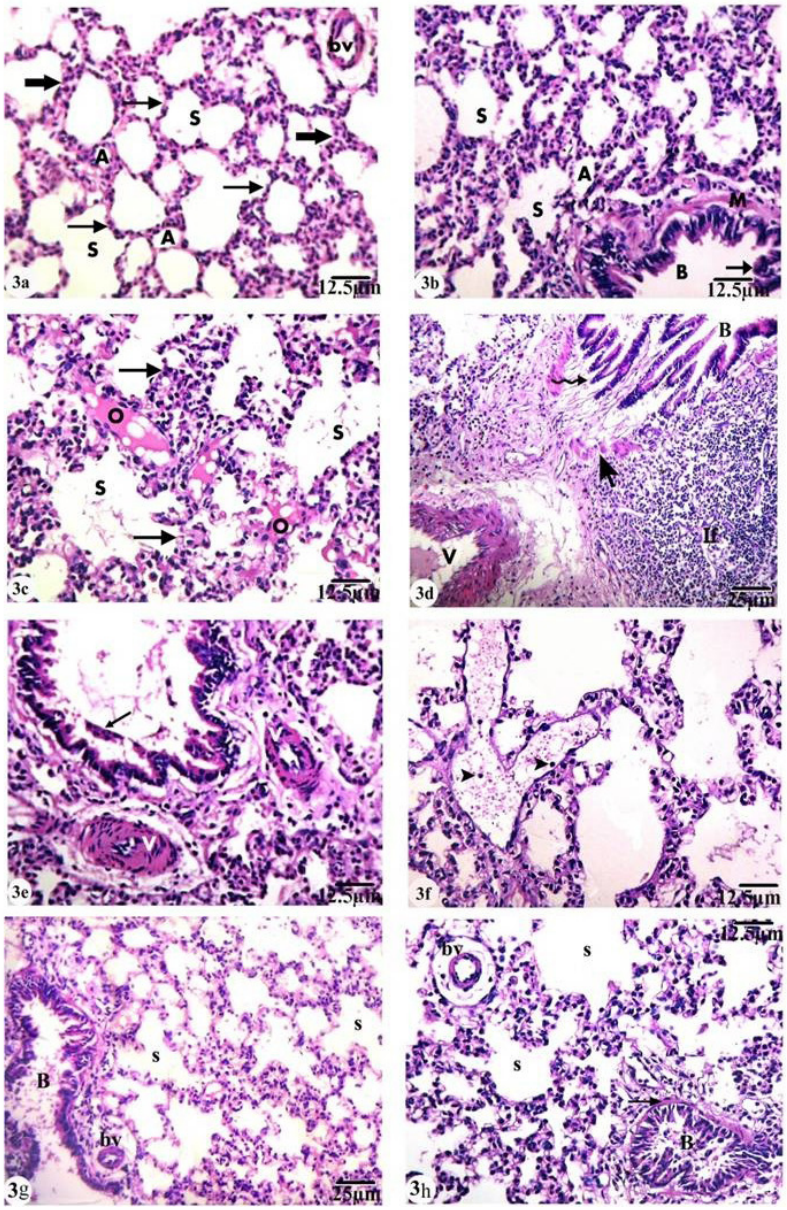

Figure 3. Photomicrographs of sections in the lung stained with H&E of control group showing (a); alveoli (A) with interalveolar septa (arrows), alveolar sacs (s) and pulmonary blood vessels (bv), (b); bronchus (B) with respiratory epithelium (arrow) and smooth muscle layer (M). Rat treated with Cd showing (c); oedema (O), air space enlargement (s), thick interalveolar septa, (d); numerous areas of aggregation of lymphocyte infiltration (If) in connective tissue surrounding lung bronchioles (B), fragmentation of the surrounding bronchial muscle layer (arrow), shedding of the mucosal lining (irregular arrow), dilatation and congestion of pulmonary vein (v), (e); some cellular debris in bronchiole (arrow), marked thickening of the wall of pulmonary vein (v), (f); deposited inflammatory cells inside alveolar sacs, (g); lung tissue of rat treated with Cd+Vit.C showing improvement similar to control and (h); lung tissue of rat treated with Cd+Zn showing partially improvement. Original magnification of (3a, b, c, e, f and h); x 200, Original magnification of (3 d and g); x 100

The histological observations on testis of control group have shown normal seminiferous tubules, spermatogenic cells and interstitial cells (Figure 4a). The rats treated with Cd have shown many histopathological changes; odema, degeneration of spermatogenic cells, pyknotic nuclei, dilatation and congestion of blood vessels. In addition to that, Cd induced a pronounced alteration of spermatogenic process with dramatically reductions of spermatozoa produced in the lumen of the seminiferous tubules sections (Figures 4b, 4c, 4d). These histopathological changes were reduced in the rats treated with Cd plus Vit.C; the spermatogenic cells were improved while the interstitial congestion was still found (Figure 4e). The rats treated with Cd plus Zn have shown partially improvement of spermatogenic cells and Pyknotic nuclei and many vacuoles were observed (Figure 1f).

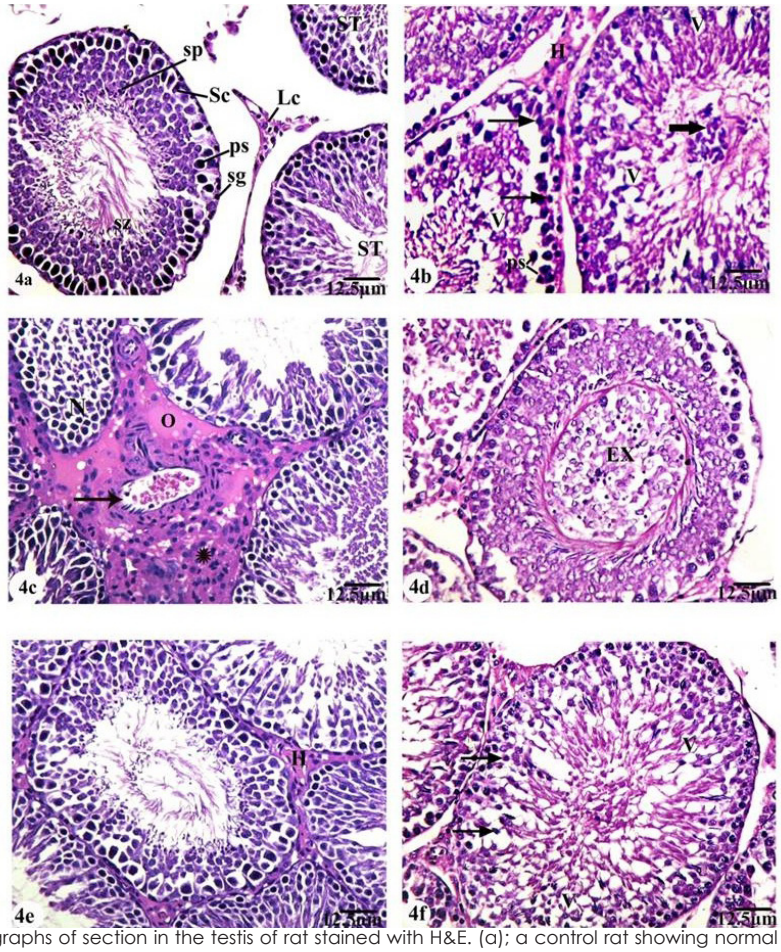

Figure 4. Photomicrographs of section in the testis of rat stained with H&E. (a); a control rat showing normal appearance of spermatogenic cells; spermatogonia (sg), primary spermatocyte (ps), spermatid (sp), spermatozoa (sz) and sertoli cells (sc). Leydge cells in interstitial tissue are noticed (Lc). Sections of rat treated with Cd showing (b); degeneration of spermatogenic cells, pyknotic nuclei (arrows), intercellular vacuoles (v), interstitial hemorrhage (H), separeation of spermatogenic cells from the basement membrane in some tubules and aggregation of little spermatozoa in lumen (thick arrow), (c); interstitial odema (O), necrosis of some tubules (N), interstitial fibroplasias with mononuclear cells infiltrations (*), dilatation and congestion of blood vessels (arrow), (d); exfoliation of cells in the lumen, (e); rats group treated with Cd+Vit.C showing restoration of spermatogenic cells in most seminiferous tubules and mild interstitial hemorrhage (H) and (f); a rat treated with Cd+Zn showing partially restoration of spermatogenic cells, many vacuoles (v), pyknotic nuclei of some spermatogenic cells (arrows). Original magnification of each figure; x 200

Azan stain

Section in the liver of control rat stained with azan revealed normal distribution of collagen fibers as a blue color in between hepatocyte, around central vein and portal area (Figure 5a). In the rats treated with Cd, the collagen fibers were increased in between hepatocyte, around central vein and portal area (Figure 5b). A marked reduction in the distribution of collagen fibers was observed to reach the normal ones in rats treated with Cd plus Vit.C or Zn (Figures 5c, 5d) respectively.

Kidney of the control rats has shown normal distribution of collagen fibers around Malpigian corpuscles and in brush border of proximal tubules (Figure 6a). In Cd-treated group, the collagen fibers were increased in glomerular tuft, in between the renal tubules and around blood vessels (Figure 6b). However, rats treated with Cd plus vit.C or Zn, a marked decrease in the distribution of collagen fibers was observed (Figures 6c, 6d), respectively.

On the other hand, the normal distribution of collagen fibers in lung sections of a control group was demonstrated around air space and bronchiole (Figure 7a). The lung tissue of rats treated with Cd exhibited increasing of collagen fibers as compact dense around bronchioles and pulmonary vein (Figures 7b, 7c). These increases were reduced in rats treated with Cd plus Vit.C or Zn and reached as in control ones (Figures 7d, 7e), respectively.

Microscopic examination of sections in the testis of control rats has shown the normal distribution of collagen fibers in tunica albuginea, basement membrane of seminiferous tubules and around blood vessels (Figure 8a). In Cd-treated animals, the distribution of collagen fibers was increased in tunica albuginea, basement membrane of seminiferous tubules and around blood vessels (Figure 8b). In rats groups treated with Cd plus Vit.C or Zn, a reduction in distribution of collagen fibers was observed (Figures 7c, 7d), respectively.

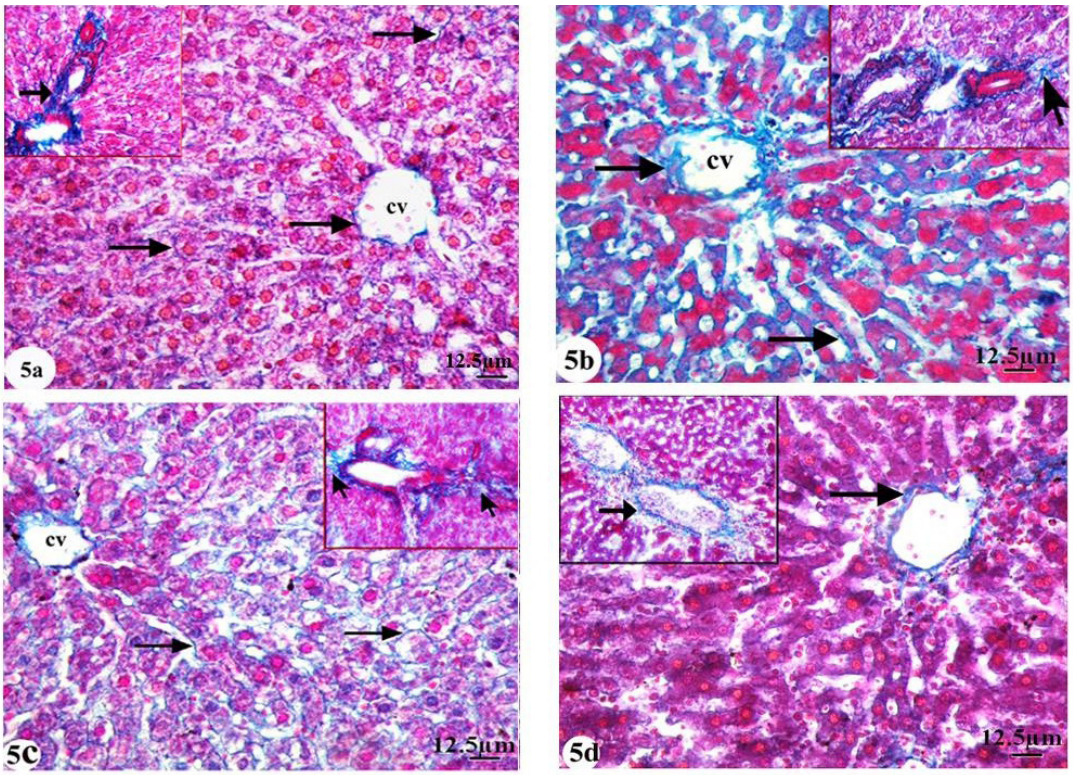

Figure 5. Sections in the liver stained with azan for demonstrating collagen fibre. (a); a control group showing minimal collagen fibres (arrows) in between hepatocyte, around central vein and at portal area, (b); rats treated with Cd showing increase of collagen fibers (arrows) in between hepatocyte, around central vein and portal area, (c & d); rats treated with Cd+Vit C and Cd+Zn respectively showing reduction in the distribution of collagen fibers similar to control. Original magnification of each figure; x 200

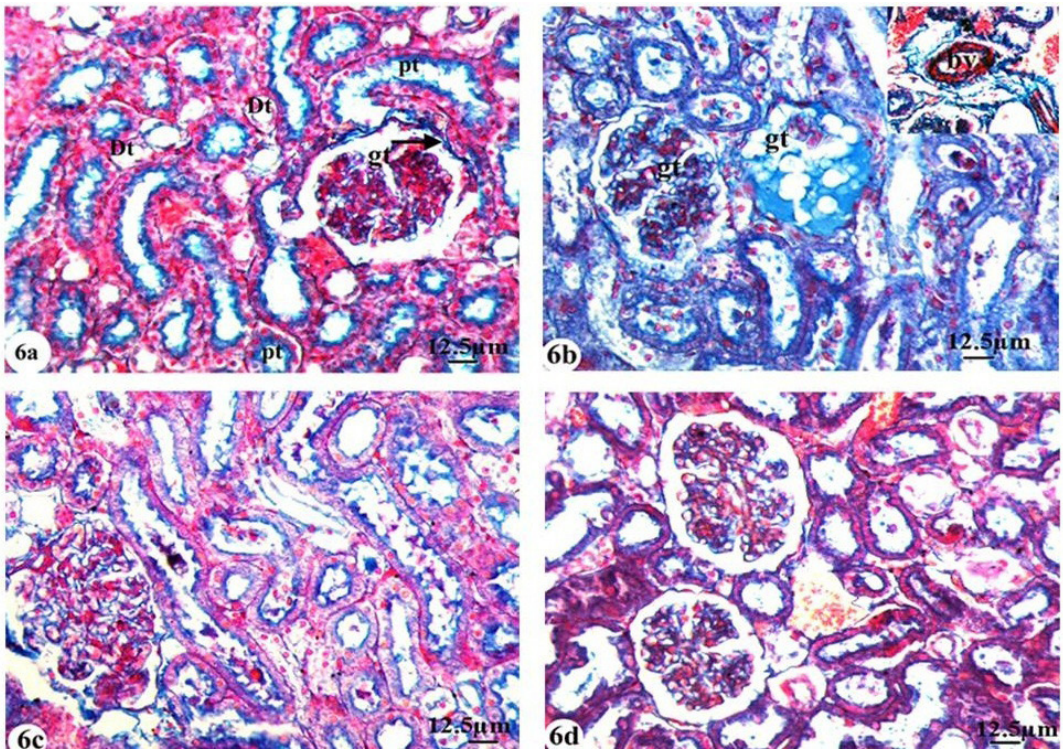

Figure 6. Sections in the cortex of kidney stained with azan to demonstrate collagen fiber. (a); showing normal distribution of collagen fibre in control group around glomeruli (arrow) and in brush border of proximal tubules (pt), (b); a rat treated with Cd showing high increase of collagen fiber in glomerular tuft, renal tubules, interstitial tissue and around blood vessels (v), (c); rats treated with Cd+Vit.C showing normal distribution of collagen fiber in brush border and around glomeruli similar to control, (d); low distribution of collagen fiber and partially improvement of brush border in a rat treated with Cd+Zn when compared with Cd treated group. Original magnification of each figure; x 200

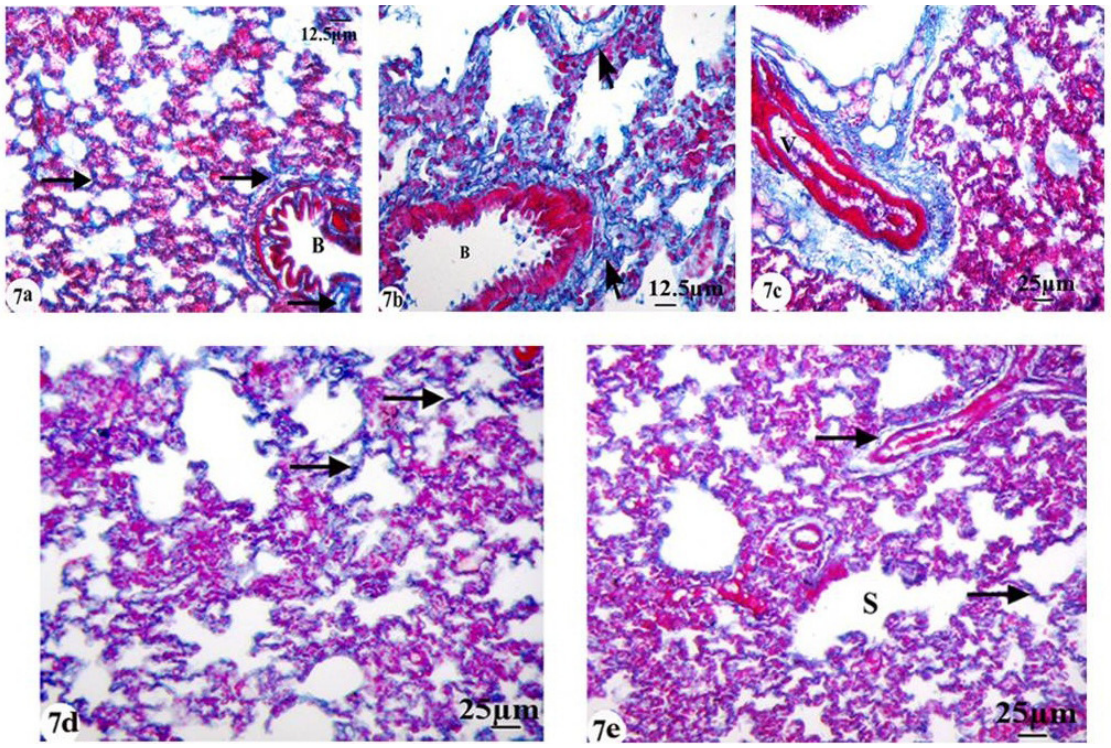

Figure 7. Sections in the lung stained with azan. (a); a control rat showing normal distribution of collagen fiber in pulmonary intersititum around bronchiole (B), alveolar sacs (s) and in between alveoli (arrows), (b & c); rats treated with Cd showing excessive deposition of collagen fiber in peribronchiolar area, thick interalveolar septa (arrows) and around dilated congested pulmonary blood vessel (v) and (d & e); rats treated with Cd+Vit C and Cd+Zn respectively showing normal distribution of collagen fibers in pulmonary intersititium around the bronchiole, alveolar sacs and around pulmonary vein (arrows). Original magnification of (7a and b); x 200, Original magnification of (7c, d and e); x 100

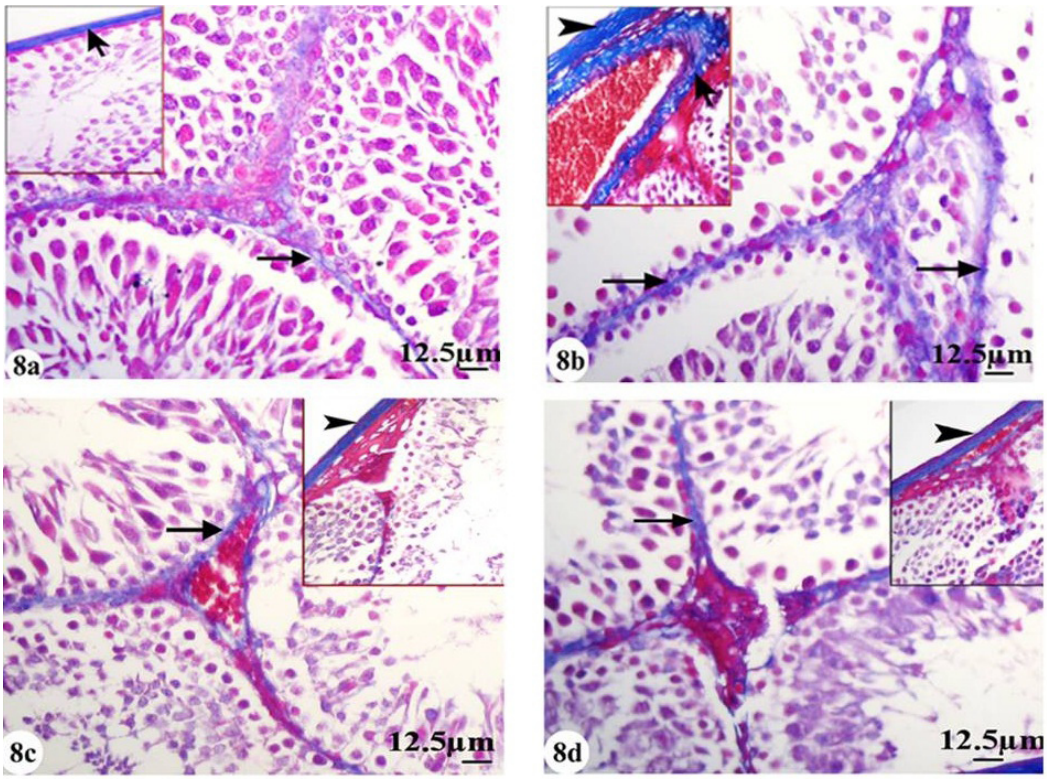

Figure 8. Sections in the testis stained with azan. (a); a control rat showing normal distribution of collagen fibers in tunica albuginea and basement membrane (arrows), (b); showing high increase in the distribution of collagen fibres in tunica albuginea (head arrow), basement membrane (long arrow) and around blood vessels (short arrow) in rats treated with Cd and (c & d); rats treated with Cd+Vit.C and Cd+Zn respectively, showing normal distribution of collagen fibers (arrows) approximately similar to control. Original magnification of each figure; x 200

Feulgen's reaction

For demonstrating DNA content, sections were stained with Feulgen's reaction to demonstrate DNA as magenta color. Sections of liver, kidney, lung and testis of the control group have shown normal DNA contents (Figure 9a, 10a, 11a &12a). The DNA content was reduced as explained by moderate magenta color in the hepatocytes of liver section and cells of renal tissue of rats treated with Cd (Figures 9b ,10b), while the lung section has shown increase of DNA content (Figure 11b). In testis of rats treated with Cd, condensed DNA content has shown shrunken spermatogonia (sign of apoptosis) while spermatogenic cells and spermatozoa exhibited weak DNA content (Figure 12b).

Rats treated with Cd plus Vit.C have shown restoration of DNA content in tissues of liver, kidney and testis similar to the control (Figure 9c, 10c, 12c) respectively; while in lung tissue, DNA content was decreased (Figure11c). Rats group treated with Cd plus Zn have shown partially restoration of DNA content in the liver and kidney sections (Figures 9d, 10d) and high restoration in testis tissue (Figure 12d). However, the lung tissue has shown a decrease in DNA content reach as control ones (Figure 11d).

The second and third groups have shown approximately normal histological and histochemical observations on tissues of liver, kidney, lung and testis of rats.

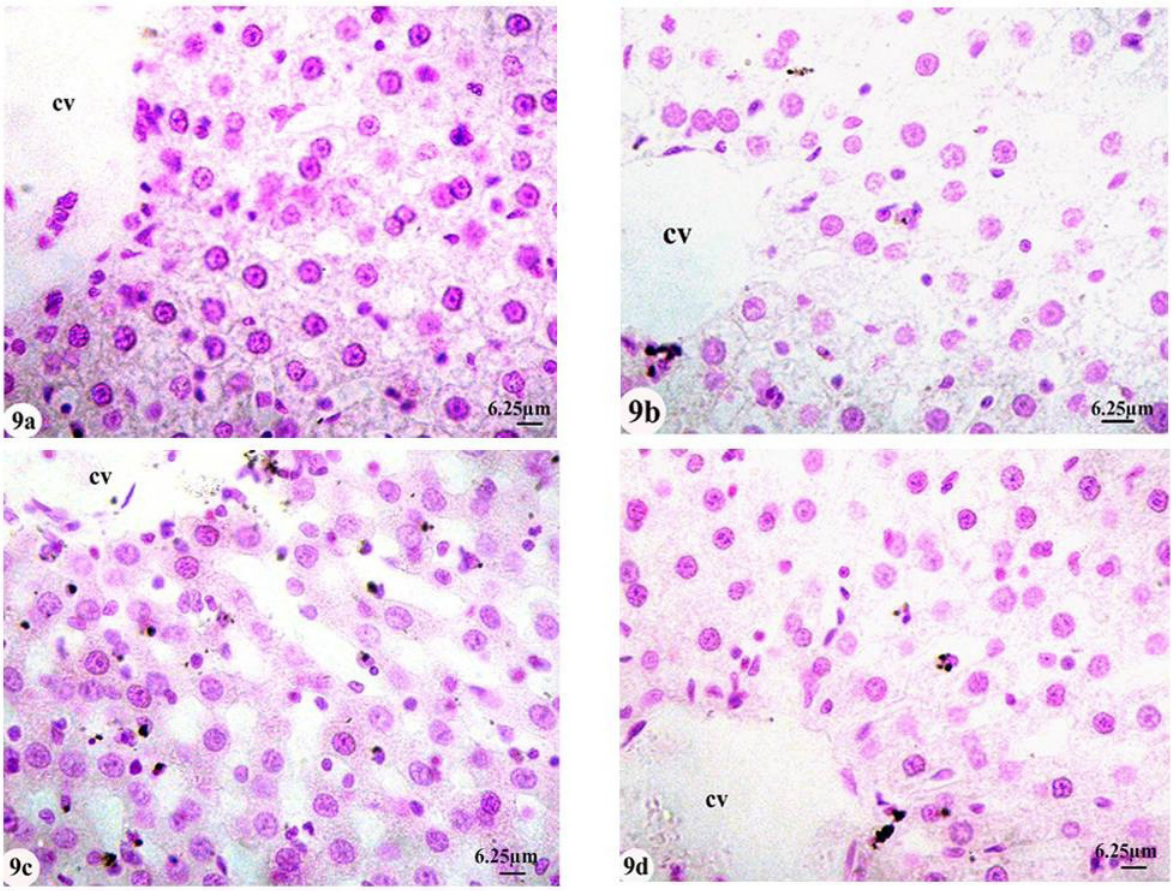

Figure 9. Sections in the liver stained with Feulgen' reaction. (a); a control rat showing normal content of DNA in nuclei of hepatocytes, (b); rat treated with Cd showing reduction of DNA content and (C & d); sections in rat treated with Cd+Vit C and Cd+Zn respectively showing partially improvement of DNA content. Original magnification of each figure; x 400

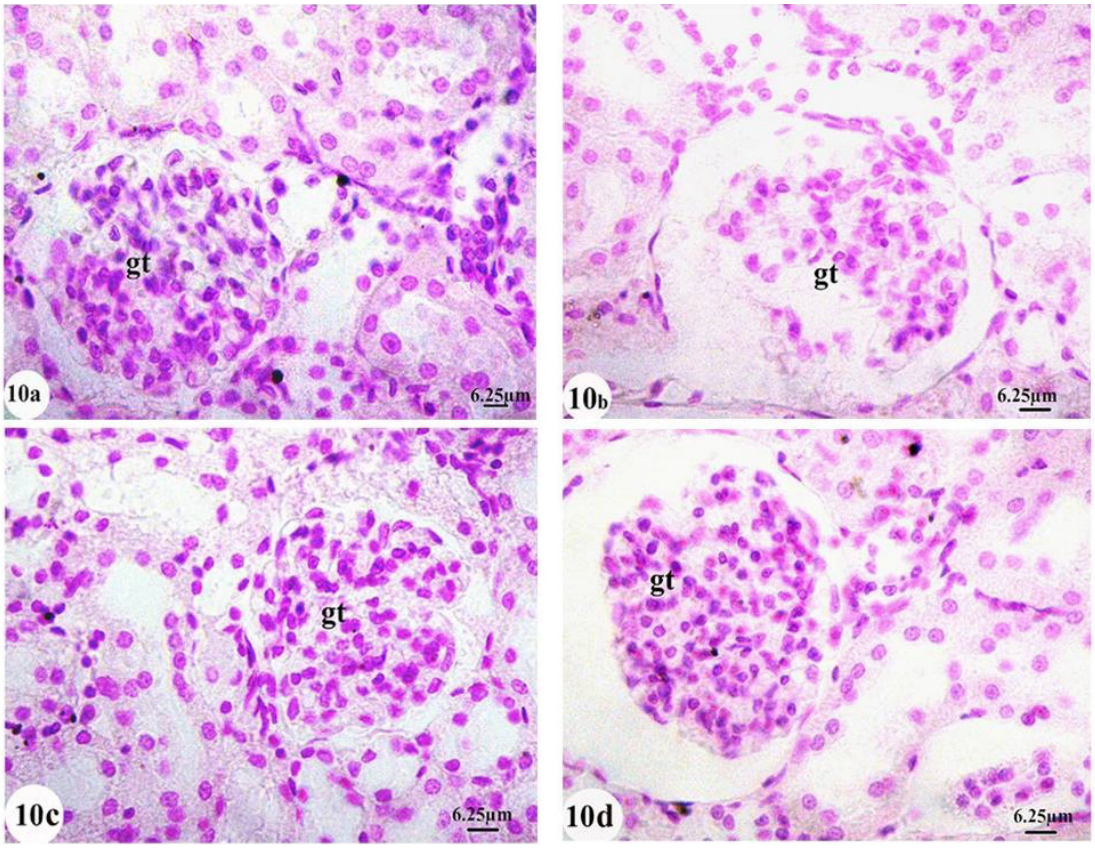

Figure 10. Sections in the kidney stained with Feulgen' reaction. (a); a control rat showing normal content of DNA, (b); rats treated with Cd showing reduction of DNA content and (c & d); sections in rats treated with Cd+Vit C and Cd+Zn showing restoration of DNA content. Original magnification of each figure; x 400

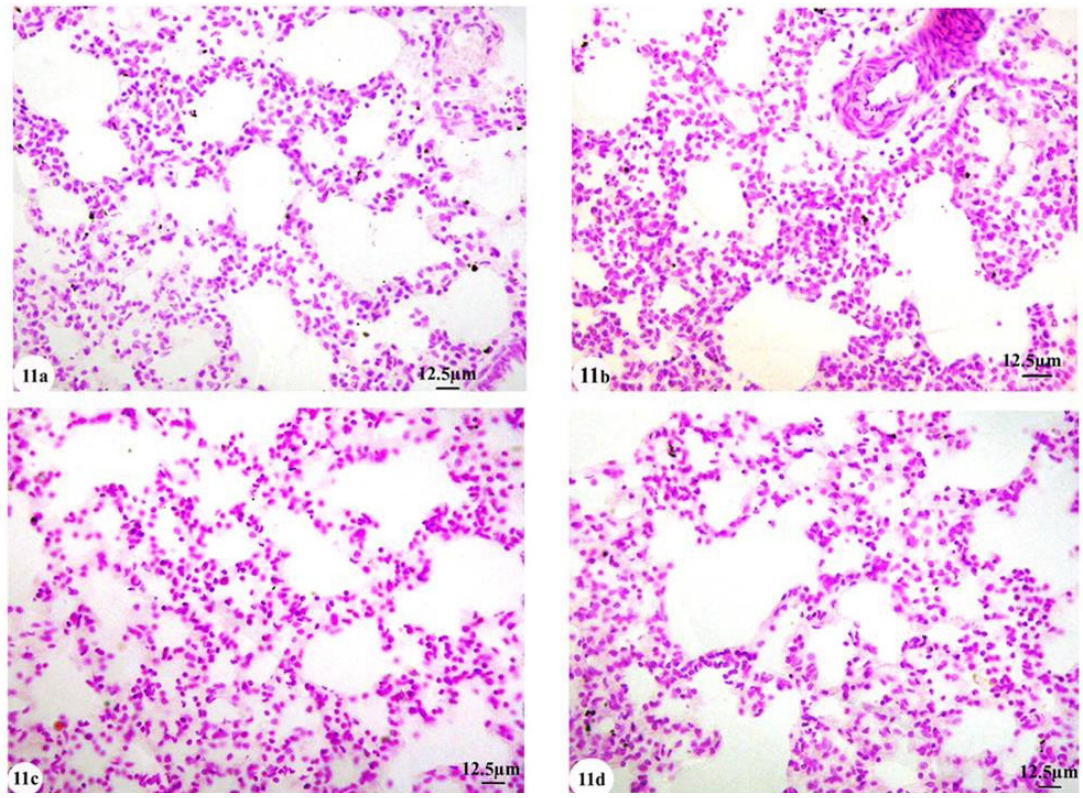

Figure 11. Sections in the lung stained with Feulgen' reaction. (a); a control rat showing normal content of DNA, (b); section of a rat treated with Cd showing increase of DNA content and (c & d); rats groups treated with Cd+Vit.C and Cd+Zn showing decrease of DNA content when compared with Cd group. Original magnification of each figure; x 200

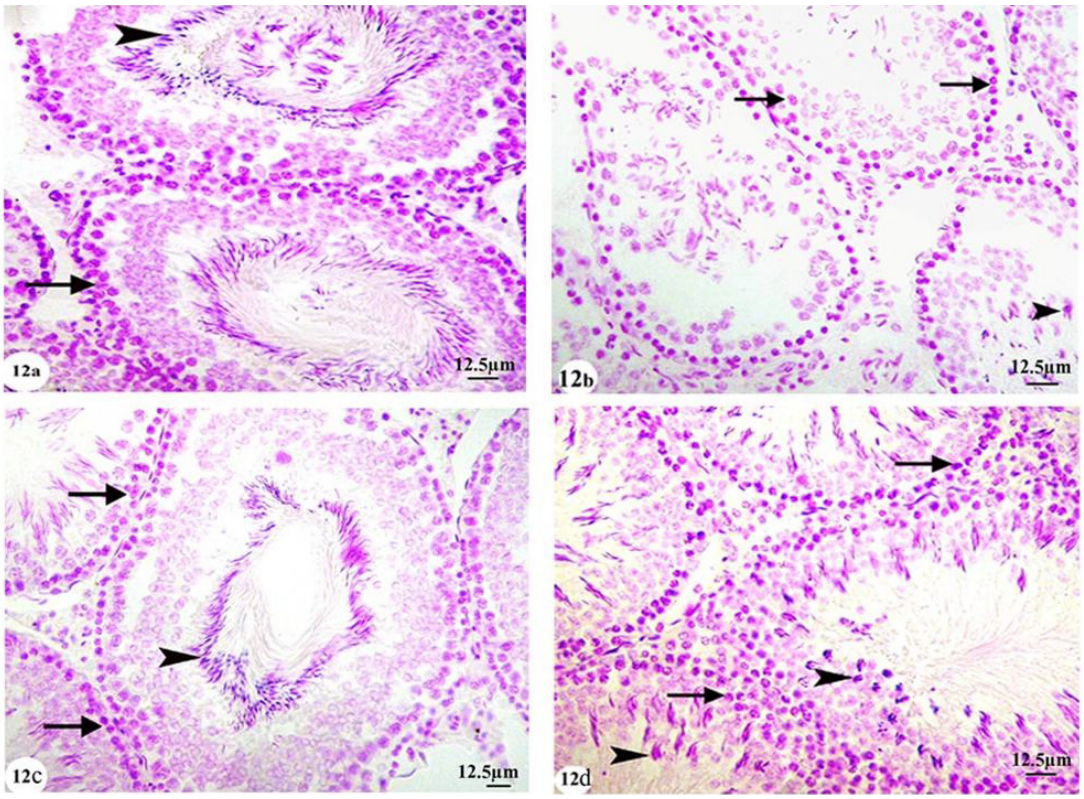

Figure 12. Sections in the testis stained with Feulgen's reaction showing (a); normal contents of DNA of spermatogenic cells (arrow) and spermatozoa (head arrow) of control rat, (b); reduction of DNA content in spermatogenic cells of a rat treated with Cd while spermatogonia appeared with condensed DNA (pyknotic nuclei) and (c & d); rats groups treated with Cd+Vit C and Cd+Zn showing restoration of DNA content. Original magnification of each figure; x 200

Cytogenetical study

Table (1) and Figure (13) summarized the results of chromosomal aberrations in bone marrow cells of male albino rats treated with Cd (3mg/kg b.w.). The chromosomal aberrations were represented in the form of chromatid deletions, dicentric, fragment, centromeric separation, ring and polyploidy. These structural and numerical types were identified and calculated relative to control. Rats treated with Cd plus Vit.C or Zn exhibited significant reduction in chromosomal aberrations induced by Cd.

The frequency of total chromosomal aberrations was significantly increased after Cd treatment for 90 days (42.5 ± 24.7) when compared with control (1.60 ± 1.1). However, the frequency of total chromosomal aberrations in rats treated with Cd plus Vit.C or Zn resulted in a significantly decrease in the frequency of chromosomal aberrations, (11.93 ± 8.33 and 13.97 ± 14.5,) respectively, if compared to Cd alone treated group.

Table 1. Chromosomal aberrations in rat bone marrow cells after treatment with cadmium chloride, vitamin C and Zn.

No. of group	Groups	Chromosomal aberrations						Total No. of aberrations	Average No. of aberration ± S.E
		R	D.C	F	C.S.	D	polyploidy		
1	Control	2	0	0	0	4	1	7	1.6±1.1
2	Vit. C	1	0	1	0	3	1	6	1.09±1
3	Zn	2	0	3	0	3	1	9	1.37±1.05
4	Cd	73	11	55	61	37	18	255	42.5±24.7*
5	Cd + Vit. C	33	3	22	29	16	7	110	11.93±8.33*
6	Cd + Zn	41	9	38	39	22	9	178	13.97±14.5*

R: ring, D.C: Dicentric, F: fragment, C.S: centric separation, D: deletion. (P<0.05) Significant Non significant (p<0.05)

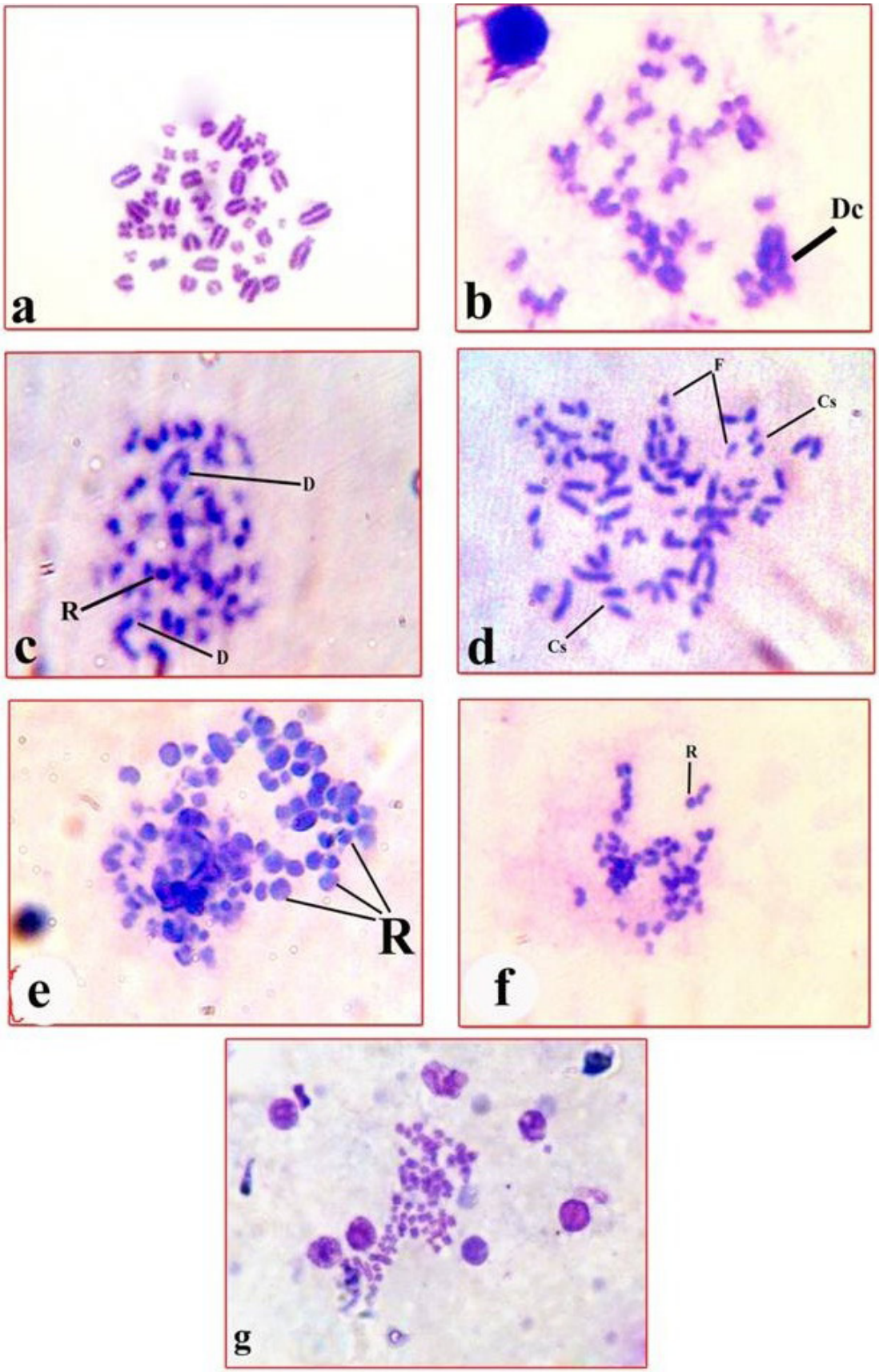

Figure 13. Metaphase figures of chromosomal aberration of bone marrow cells showing: (a); normal metaphase, (b); Dicentric (Dc), (c); Deletion (D) and ring (R), (d); fragment (F) and centric separation (cs), (e & f); Ring (R) and (g); polyploidy. Original magnification of each figure; x 1600

Discussion

Cadmium has been demonstrated to stimulate free radical production, resulting in oxidative deterioration of lipids, proteins and DNA, and initiating various pathological conditions in humans and animals (Shaikh et al., 1999; El-Demerdash et al., 2004). Cadmium exposure, acute as well as chronic, is associated with elevated lipid peroxidation in various tissues such as lung, brain, kidney, liver, erythrocytes and testes (Manca et al., 1991; Sarkar et al., 1997).

The liver is the primary target organ following acute systemic Cd exposure. The uptake of Cd into the liver is critical for the development of overall toxicity induced by heavy metal. Approximately half of Cd absorbed systemically is rapidly accumulated in the liver, which resulted in the reduced availability of Cd to such organs as the kidneys and testes, which are more sensitive to its toxic actions (DelRaso et al., 2003).

In the present work, the administration of Cd resulted in severe hepatocyte necrosis, fatty changes, degeneration signs and inflammatory cell infiltrations. These result is similar to those reported previously in the literature (Gong et al., 2008; Ersan et al., 2008; Renugadevi & Prabu, 2010). Parenteral administration of a soluble Cd salt in rats causes a rapid accumulation of Cd in the liver. Hepatic necrosis has been reported in rats and mice after acute exposure to Cd (Dudley et al., 1982; Theocharis et al., 1991; Krichah et al., 2003). Subchronic exposure to $CdCl_2$ caused liver damage, demonstrated by histopathological alterations; moderate degeneration (ballooning) and a discrete necrosis (Borges et al., 2008). The necrosis was mostly centrilobular and extending through the whole liver lobule (Sinha et al., 2009). The histopathological changes of liver treated with Cd might be due to the formation of highly reactive radicals and subsequent lipid peroxidation induced by Cd. The accumulated hydroperoxidase can cause cytotoxicity, which is associated with the peroxidation of membrane phospholipids by lipid hydroperoxidase, the basis of hepatocellular damage (Renugadevi & Prabu, 2010).

The nephrotoxicity especially glomerulus and tubular changes; shrunken and degenerated of glomeruli; pyknosis and vacuolated cytoplasm of tubules were observed after treatment with Cd in the present work. This result is in accordance with that reported by Jemai et al. (2010), who have found that Cd affected the glomeruli especially glomerular capillaries in favour of Bowman's space, atrophy of some glomerulus. Several histopathological studies revealed the toxic effect of Cd in the kidney; edema was usually found (Choi & Rhee, 2003), as well as proximal tubular necrosis, apoptosis, and tubular degeneration (Damek-Poprawa & Sawicka-Kapusta, 2004).

Cadmium-induced nephrotoxicity is thought to be mediated through the cadmium metallothionein (Cd-Mt) complex, which is synthesized in the liver, released into circulation and taken up by renal proximal tubule cells (Dudley et al., 1985). In fact, when the synthesis of Mt becomes insufficient for binding all Cd ions in the liver, the free Cd ions, which do not bound to Mt, produce hepatocyte injury and a Cd-Mt complex is released into the blood stream. The complex in the plasma is then filtered through the glomeruli in the kidney and taken up by the proximal tubular cells (Sudo et al., 1996). On its way through the kidney, this complex causes injury, mainly in the cortical region, reaching the proximal tubule and causing a gradual loss of the organ's function (Dorian & Klaassen, 1995; Thijssen et al., 2007). Moreover, these changes may be due to the accumulation of free radicals as the consequence of increased lipid peroxidation by free Cd ions in the renal tissues of Cd-treated rats (Renugadevi & Prabu, 2009).

The lung tissue of rats treated with Cd in the present study revealed severe inflammation and cell proliferation. The chronic exposure to cadmium compound induces lung cell proliferation, what may be independent of lung inflammation (Kundu et al., 2009). These authors hypothesized that cadmium exposure induces the inflammatory cytokines along with the cell proliferating factors in the lung.

Recently, El-Sokkary & Awadalla (2011) have reported different changes in the lung of rats treated with Cd at a dose of 5 mg/kg bw. Lung lesions are consisting of vascular severe inflammation in both alveoli and bronchioles with edema and congestion. Also, these histopathological changes are in agreement with the findings of Shin et al. (2004), who have reported that the lung is a primary target organ of systemic exposure to Cd. Cadmium is mainly absorbed through the inhalation of industrial pollution and tobacco smoke, resulting in the accumulation of this metal in the lung. Yamada et al. (1982) have noticed a dramatic increase in the number of alveolar neutrophilic leukocytes 6-48 h after intra-bronchial instillation of 1 mg $CdCl_2$ into lungs of dogs. However, Driscoll et al. (1992) have reported that inhalation of Cd has been implicated in the development of emphysema and pulmonary fibrosis. Mckenna et al. (1997) have found that Cd-exposed lungs showed acute and more chronic pulmonary inflammation in both rats and mice with bronchiolar and alveolar lesions. Cd exposure was deleterious to the lung tissue causing mild to severe inflammation (Bell et al., 2000).

In agreement with the results of EL-Shahat et al. (2009), the current study has shown marked histological changes in the testis tissue in the form of degeneration of spermatogenic cells, oedema, haemorrhage, congestion, and multifocal areas of ischemic necrosis. However, Blanco et al. (2007) have claimed that even with low doses of $CdCl_2$ (1mg/kg bw for one month) lack of spermatogenesis and severe necrosis of the testes

of rats were induced. Moreover, Santos et al. (2004) have reported that endothelial damage of the small blood vessels, oedema and hemorrhage of the rat testes can be demonstrated by using just a single parenteral dose of $CdCl_2$ at 2-4 mg/kg bw. Several studies focusing on Cd-related changes in testicular histopathology have implicated testicular blood vessel damage, followed by the degeneration of spermatopoietic epithelial, as the main cause of Cd toxicity (Thompson & Bannigan, 2008; Messaoudi et al., 2010).

The nature and degree of testicular damage is Cd dependent. More than 10 µmol/kg dose of Cd causes severe testicular lesions, such as hemorrhagic necrosis in rats and mice and interstitial cell tumor in rats (Waalkes, 2000). Moreover, lower dose Cd (5 µmol/kg or less) induces apoptosis in spermatogenic cell-sensitive to Cd (Zhou et al., 1999).

In the present work, Cd administration exhibited increment of collagenous fibers in the tissues of liver, kidney, lung and testis of rat. This agrees with Faeder et al. (1977), who have stated that administration of Cd at 0.2, 0.5 or 0.75 mg/kg bw. for 8 weeks exhibited proliferation of prominent connective tissue fiber bundles.

Histochemically, Cd-treated rats exhibited decrease of DNA contents in the tissues of liver, kidney and testis while the lung tissue exhibited an increase in DNA contents. Cd induced random fragmentation of genomic DNA (Yang et al., 2003). The enzyme thymidine kinase (TK), responsible for the phosphorylation of deoxythymidine and its subsequent incorporation into DNA, has been involved in the inhibition of DNA synthesis in Cd-treated cell cultures (Andranovich et al., 1985). The enzyme TK is inhibited in the liver of Cd-treated rats (Theocharis et al., 1992) and the increase of DNA content in lung tissue may be due to the high increase of inflammatory cells

Cytogenetically, Cd induced chromosomal aberrations in the present work; deletion, ring, centric separation and dicentric. Chandra & Khuda-Bukhsh (2004) have revealed that $CdCl_2$ induced aberrations of various kinds in *O. mossambica,* such as break, terminal association, centric fusion, precocious centromeric separation and C-mitosis. Cd had already been reported to have adverse effects on chromosomes and Cd burden in the body has been reported to be directly correlated with chromosomal aberrations (IARC, 1993). Low dose of cadmium (1mg/kg/day) for 30 days resulted in chromosomal aberrations as manifested by aneuploidy, breaks, gaps, centromeric fusion resulting in formation of submetacentric and metacentric chromosomes. However, it was also observed that $CdCl_2$ administration at a large dose given staggeredly eg. 25mg/kg/day for 20 days and 200mg/kg/day for 5 days resulted in severe chromosomal damage if compared to the same dose given singly (Singh et al., 2007). Singh & Sankhla (2010) have observed that in animals administered with $CdCl_2$ there was a significant increase in the number of chromosomal aberrations and a decline in mitotic index. Free-radicals can originate from exposure to various environmental toxicants, cadmium being one of them, resulting in disturbed homeostasis and induction of biological stress as manifested by a sharp decline in mitotic index and an elevation of chromosomal aberrations.

Vitamin C (ascorbic acid) is the major non-enzymatic antioxidant which has synergetic action in scavenging oxygen-derived free radicals and this vitamin is likely to be most susceptible to free radical oxidation (Renugadevi & Prabu, 2010). The present study has demonstrated that cotreatment with Vit C ameliorated histopathological damage induced by Cd in tissues of liver, kidney, lung and testis. Vit C plays a beneficial role in reducing the toxicity and absorption of Cd. Moreover, the influence of ascorbic acid on Cd uptake varied with the various organs (Grosicki, 2004). The beneficial effect of supplemental Vit C may be the improvement of iron absorption from the gastrointestinal tract by reduction of Fe^{+3} to Fe^{+2} and by formation of stable chelates with iron. This positive effect of Vit C on iron absorption is very desirable, since Cd is known to decrease dietary iron absorption (Maji & Yoshida, 1974). Earlier studies on the beneficial role of Vit C on Cd-induced organ toxicity have reported that antioxidant supplements with Vit C play prophylactic effect in those pathophysiological situations (Koyuturk et al., 2007; Acharya et al., 2008).

Vit C protects DNA against damage induced by reactive oxygen species (Duthie et al., 1996). The combined effect of Cd and Vit C was stronger than the sum of the effects of the compounds applied separately, so a synergistic induction of DNA damage by Cd and Vit C can be assumed (Blasiak et al., 2000). Moreover, it was reported that Vit C had a protective role against mercury-induced genotoxicity, which is probably due to its strong antioxidant and nucleophilic nature (Rao et al., 2001). In the present work, Vit C exhibited reduction in the chromosomal aberrations induced by Cd. The ability of Vit C to minimize the incidence of chromosomal aberrations induced by $CdCl_2$ in cultured mouse spleen cells had been reported by Fahmy & Aly (2000).

It is well known that Zn is an essential component of the oxidant defense system with participation at multiple cellular levels (Bray & Bettger, 1990). As shown in the present work, treatment with Cd and Zn partially protected against the damage in liver, kidney, lung and testis tissues. Liu et al. (1998) have demonstrated that Zn has a protective effect on histological damage by maintaining membrane integrity due to its direct action on free radicals. Moreover, the main reason for the restorative action of Zn against Cd toxicity may be due to an interaction between

two cations in absorption and distribution phases. Enhanced consumption of Zn may decrease Cd absorption from the digestive tract and its accumulation in the organism and as a result, it may ameliorate the toxic effects of Cd (Brzoska & Moniuszko-Jakoniuk, 2001). Jihen et al. (2009) have documented the partial ameliorative effects of Zn on Cd- caused depletion in antioxidant enzyme activities in rat liver. Zn induced the synthesis of metallothionein, a cadmium binding protein, which is widely implicated in the sequestration of this metal, and as a result it may prevent the toxic effects of Cd, including mainly the kidney damage (Jemai et al., 2010). Furthermore, Liu et al. (1998) have demonstrated that Zn prevents the nephrotoxic impact of Cd.

Zn administration minimized oxidative damage and reversed the impairment of spermatogenesis and testosterone production induced by Cd in the rat testis (Amara et al., 2008). Moreover, Zn protects against Cd-induced testicular damage essentially by preventing Cd accumulation and Zn deprivation and by ameliorating the testicular antioxidant status (Messaoudi et al., 2010). The addition of $ZnSO_4$ (1mM) protected the cells and prevented the degradation of the DNA induced by the Cd. Zinc may prevent apoptosis by inhibiting the Ca^{2+}/ Mg^{2+}-dependant endonuclease (Walton et al., 1993) and the apoptotic protease, the caspase 3 (Perry et al., 1997) or by promoting DNA synthesis and the anti-apoptotic proteins Bcl-2 activation (Ishido et al., 1999).

Conclusion

In conclusion, the present study demonstrated that both Vit C and Zn have a protective role against Cd-induced histological changes in liver, kidney, lungs and testis, as well as the cytotoxicity in bone marrow in rats. This protection was more effective in the group treated with Cd plus Vit C if compared to those treated wit Cd plus Zn. Consequently, Vit C may be applied as a protective agent for people highly exposed to Cd.

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