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GENOTOXIC AND HISTOPATHOLOGICAL EFFECTS OF CADMIUM IN MALE SWISS ALBINO MICE, *MUS MUSCULUS*

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Spermatids
Interstitial cells of Leydig
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Hepatocytes
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ABSTRACT
Oral administration of cadmium chloride caused DNA damage in the tissues of testes, liver and kidneys. Marked changes in the histological architecture of these organs were observed. Distortion of seminiferous tubules, desquamation of spermatogonia, spermatocytes and spermatids with cytoplasmic vacuolization and nuclear fragmentation in testes; disruption in arrangement of hepatic plates, widespread fibrosis, interstitial mononuclear cellular infiltration and hemorrhagic spots with necrotic hepatocytes having pycnotic nuclei in the liver and severe multifocal cloudy, hydropic degeneration with loss of cellular integrity in the renal tissues were observed. It was observed that the severity of pathological effects was dependent on the duration of post treatment period.

INTRODUCTION

Heavy metals occur naturally and many of them are essential components of our ecosystems. Heavy metals, depending on their oxidation states can be highly reactive and as a consequence, toxic to most organisms even at low concentration. Environmental pollution by metals has become extensive as mining and industrial activities increased in past few decades (Kennish, 1996). These pollutants, which are derived from growing number of diverse anthropogenic sources, industrial effluents, urban run-off, sewage treatment plants, agricultural runoff and domestic garbage dumps etc., have progressively affected more and more ecosystems (Cotte-Krief *et al.,* 2000; Bu-Olayan *et al.,* 2001; Macfarlane and Burchett, 2001 and Esser and Volpe, 2002).

Heavy metals induced cytopathological and hematological effects in various organs of lower vertebrates like fishes have been studied by many scientists (Mc Dowell, 1992; Gupta and Sharma, 1994; Sharma and Sharma, 1994; Srivastav and Sharma, 1996; Singh and Gaur, 1997; Gurer-Orhan and Ercal, 2000; Srivastava and Kaushik, 2001; Shukla *et al.,* 2002; Gupta and Srivastava, 2006; Phetsombat *et al.,* 2006 and Babu *et al.,* 2007). Unfortunately, relatively few studies have systematically addressed the impact of heavy metals on mammals in general (Buchancova *et al*., 1994; Staessen *et al.,* 1996; El-Zohairy *et al.,* 1996 and Sram *et al.,* 1996). What limited data that does exist suggests that exposure to heavy metals results in disruption of reproductive abnormality in mammals (Kempinas *et al*., 1994 and Mohapatra *et al*., 2011). Doreswamy *et al*. (2004) studied the effect of nickel on histoarchitecture of testis, lipid peroxidation in testis and epididymal sperm, damage of DNA, induction of apoptosis in testis and incidence of abnormality of sperm head in mice. Sharma *et al*. (1980) in guinea pig and Yang *et al*. (2006) in rats showed that administration of $CdCl_2$ caused a decline in the weight of the body as well as of the testes, degeneration of germinal epithelium accompanied by nuclear pycnosis followed by cytolysis and nuclear fragmentation in seminiferous tubules. Mohapatra *et al*. (2009 a,b) observed the histoarchitectural changes in the stomach and small intestine of mice exposed to arsenic and cadmium.

Testicular tumors are common in rats injected with cadmium (Shiraishi and Walkees, 1996; Walkees and Misra, 1996 and Waalkes *et al*., 1999). In humans, the seminal plasma and blood plasma cadmium concentration is higher in infertile men than fertile men which affects semen volume, sperm density and causes teratozoospermia (Chia *et al*., 1992 and Xu *et al.,* 1993). The reports available though indicate an imbalance in reproductive homeostasis of mammals exposed to heavy metals; studies on genotoxicity and histopathology of mammalian tissues are scanty. The paper deals with the genotoxic and histopathological study on the effects of cadmium in Swiss Albino mice, *Mus Musculus*.

MATERIALS AND METHODS

Adult Swiss albino mice of ten weeks old were kept in groups of six in polypropylene cages (Tarson India Ltd.) under standard conditions. They were maintained on commercial mice feed, pallets, supplemented with germinated grams and

vegetables and had free access to water. The cages were housed under normal light and dark conditions in a controlled atmosphere with a temperature of 25ºC ± 5ºC and a mean relative humidity of 50% ± 5%.

To study the genotoxic and histopathological effects of cadmium, a single dose of cadmium chloride dissolved in distilled water (3g per kg body weight) were administered orally to male mice with the help of a micro-pipette. The control groups were also maintained side by side. Control group mice received equal volume of distilled water. Mice from both control and treated groups were killed after 48h and 96h of treatment. Body weights were recorded on the days of treatment and autopsy of both control and treated groups.

The dissections were carried out in normal saline. For genotoxic studies, testes and liver were fixed in Carnoy's fixative, embedded in paraffin wax, sectioned on a microtome apparatus. The sections were stained by Feulgen's staining technique. For histopathological studies, testes, liver and kidney were fixed in Bouin's fixative, dehydrated, infilterated in melted paraffin (60ºC), embedden on paraffin blocks and sectioned perpendicular to the long axis of the tissues at a thickness of 5μm on a microtome apparatus. The tissues then were rehydrated and stained with Ehrlich hematoxyline and eosin (Adam and Caihak, 1964). Stained sections were then mounted on glass slides with Dextran Plasticizer Xylene (DPX) and covered with a cover slip. Histomorphological and genotoxic changes were evaluated using light microscope and photographed using a digital camera.

RESULTS

Effects on testis

The histological sections of the control group testes showed normal histoarchitecture having tunica, seminiferous tubules lined by basal lamina and little connective tissues surrounding tubules containing interstitial cells of Leydig and blood vessels (Fig. 1, 2). Seminiferous tubules showed normal spermatogonia (stem cells) which are in direct contact with epithelial basal lamina, diploid primary spermatocytes, haploid secondary spermatocytes and spermatids with sperms in the lumen (Fig. 1, 2). Also in the tubules are present large sized sustentucular cells or sertoli cells. There were marked changes in testicular histology of cadmium treated mice relative to control group. A disorganization of the spermatogonial cells was observed in the treated mice to different extent in 48h and 96h of post-treatment. These cells are observed to be a bit detached from the epithelial basal lamina of seminiferous tubules (Fig. 3-6). Various tubular cells appear desquamated with disintegration of cytoplasm evident by presence of vacuoles and paler staining. Nuclei of majority of cells inside seminiferous tubules exhibited nuclear pycnosis or nuclear fragmentation which is more pronounced in 96h post-treatment mice (Fig. 5, 6). Marked cytoplasmic vacuolization in sertoli cells, reduction in size of Leydig cells and necrosis of connective tissue in the interstitial spaces were observed in treated mice. There is pronounced decrease in number of sperms in the lumen of seminiferous tubules. In 96h post-treatment mice, some tubules were totally devoid of sperms (Fig. 5, 6). Almost all the types of cells in seminiferous tubules of treated mice in comparison to control mice showed light pink staining of their nuclei after Feulgen's reaction, suggesting that the DNA has probably degraded (Fig. 7-12).

Effects on liver

Histological sections of liver of control group mice showed normal liver lobules containing plates of hepatocytes radiating out from central veins (Fig. 13, 14). Between the hepatocyte plates present sinusoids. Application of heavy metal under investigation induced many pathological alterations in the hepatic tissues. Tissue sections of treated mice manifested disorganized structure evident from disruption of normal hepatocyte plates or cords arrangement. The nuclei of hepatocytes appear small sized with disintegrated chromatin in comparison to that of control mice. In the liver sections of 96 hours post-treatment mice, cytoplasmic vacuolization of the hepatocytes were more severe (Fig. 17, 18). Nuclear pycnosis was also evident in such cells. In many sections, the hepatocytes have become completely disrupted, cell membrane were ruptured, nuclei showed no sign of having nucleoli or chromatin (Fig. 15-18). A clear space around the nuclei of these hepatocytes has been noticed due to cytoplasmic vacuolization. Widespread fibrosis, interstitial mononuclear cellular infiltration and hemorrhagic spots were observed in mice exposed to cadmium. Increased intercellular spaces in the hepatic lobules due to necrosis were severe in 96h post-treatment than 48h post treatment mice. Nuclei of hepatocytes of treated mice stained lighter in comparison to control group after Feulgen's reaction indicated degradation of DNA (Fig. 19-24).

Effects on kidney

No histological or macroscopic alterations were observed in the kidneys of control mice. There was normal arrangement of renal corpuscles, proximal and distal convoluted tubules, Henle's loop and collecting duct (Fig. 25, 26). The histoarchitectural changes observed in the cadmium treated mice were lesions in the cortex and medulla. These consisted of mild to severe multifocal cloudy and hydropic degeneration with necrosis in the tubule. There was significant distortion of Bowman's capsules with disorganized glomeruli in the 96h post-treatment mice (Fig. 29, 30). The cubical epitheliums of proximal and distal convoluted tubules were maximum affected showing loss of cellular integrity (Fig. 29, 30). Majority of the cells had paler cytoplasm due to vacuolization with disrupted nuclei indicated by the presence of scanty chromatin which is more pronounced in 96h post-treatment mice. It was observed that the severity of the pathological effects was dependent on the duration of post treatment period. The nuclei of various cells in cortex and medulla of kidney of control mice stained dark pink after Feulgen's reaction Fig. 31, 32). Light pink staining of the nuclei in the tubular cells and collecting ducts of treated mice after Feulgen's reaction indicated the possibility of DNA damage by cadmium (Fig. 33-36).

DISCUSSION

Heavy metals are known to exert toxic effects on multiple organs in different animals. These toxic effects are due to disturbances of the normal gene expression in the tissues.

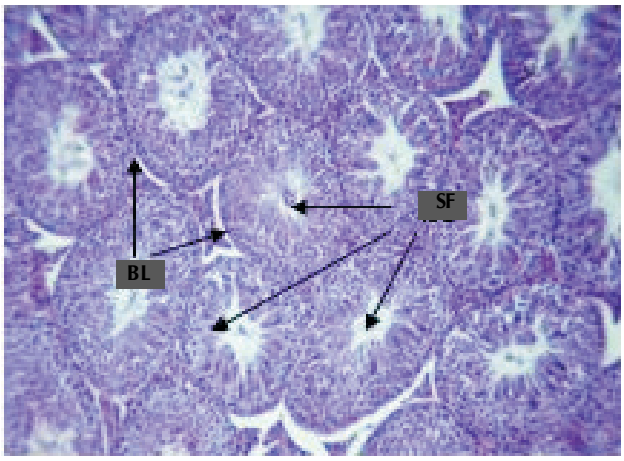

Figure 1: Section of testis of control mice showing normal histoarchitecture containing seminiferous tubules (SF) covered by basal lemina (BL). 100x

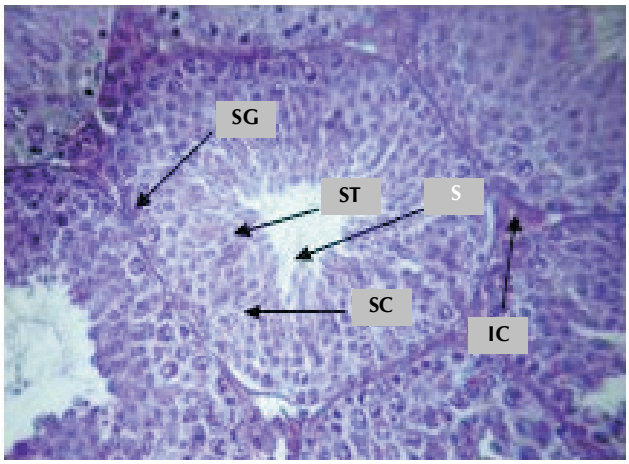

Figure 2: Section of testis of control mice showing spermatogonia (SG), spermatocytes (SC), spermatids (ST), sperms (S) and interstitial cells (IC). 400x

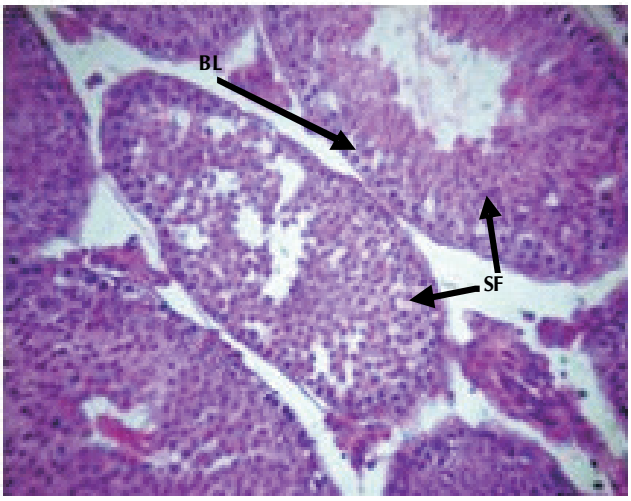

Figure 3: Section of testis of 48 hours post-treatment treated mice showing disorganization of cells in SFs. 400X

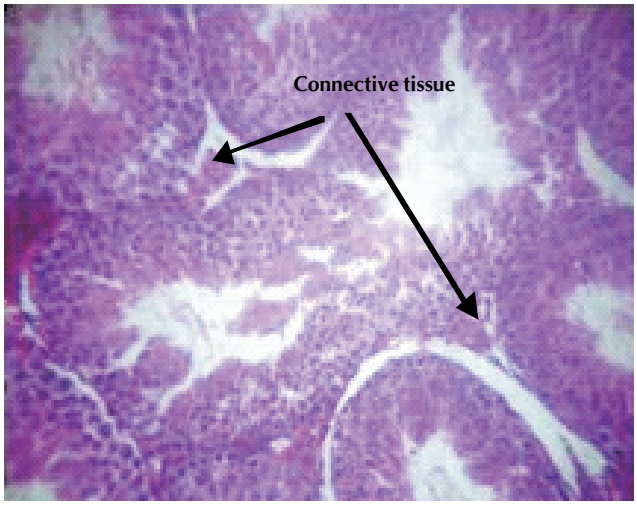

Figure 4: Section of testis of 48 hours post-treatment of treated mice showing necrosis of tissues in the interstitial spaces. 400X

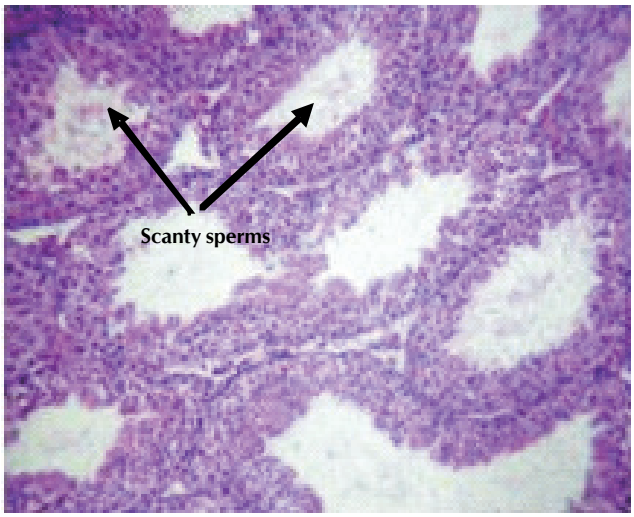

Figure 5: Section of testis of 96 hours post-treatment treated mice showing showing disorganization of cells and devoid of sperms in SFs. 400X

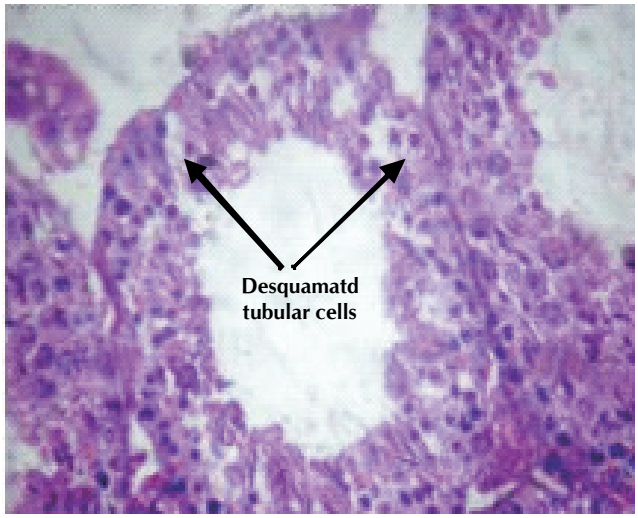

Figure 6: Section of testis of 96 hours post-treatment of treated mice showing cytoplasmic vacuolization cells in SFs. 400X

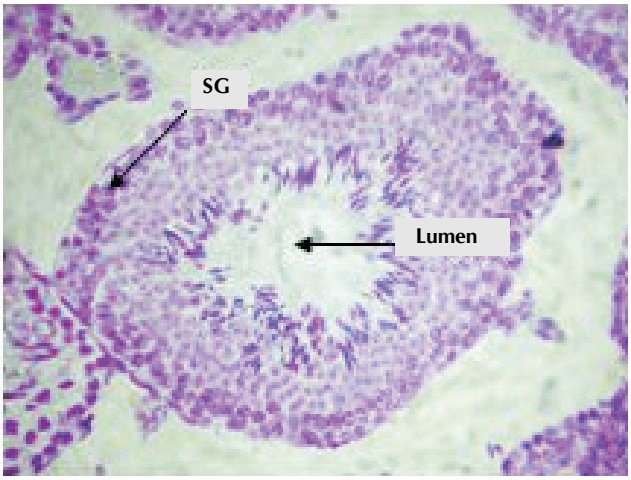

Figure 7: Section of testis of control mice showing DNA content in the cells of semiferous tubule (SF) after Feugen's reaction. 400X

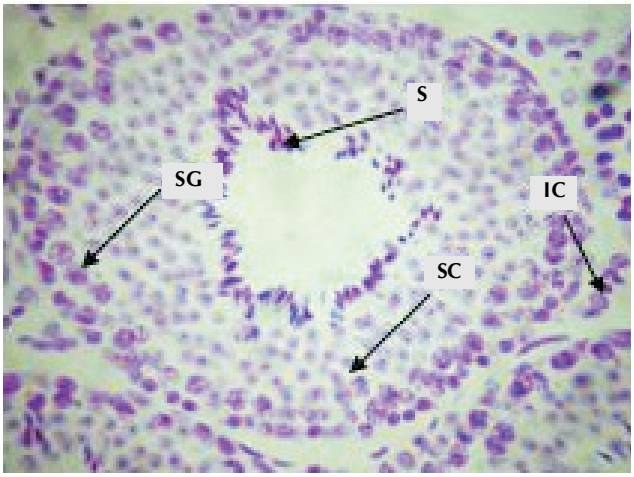

Figure 8: Section of testis of control mice showing DNA content in the SGs, SCs, STs and in sperms after Feulgen's reaction. 600X

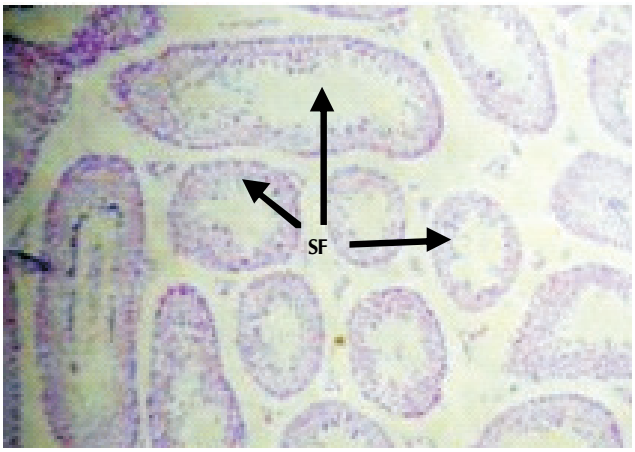

Figure 9: Section of testis of 48 hours post-treatment mice showing DNA content in the cells of semiferous tubules (SFs) after Feulgen's reacion. 100X

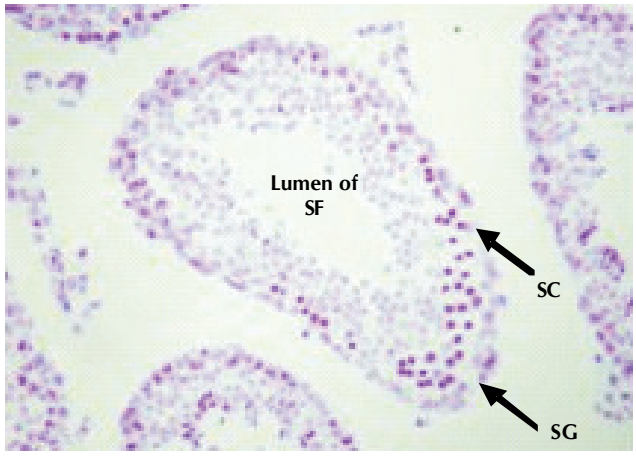

Figure 10: Section of testis of 48 hours post-treatment mice showing DNA content in the SGs, SCs, STs and in sperms after Feulgen's reacion. 400X

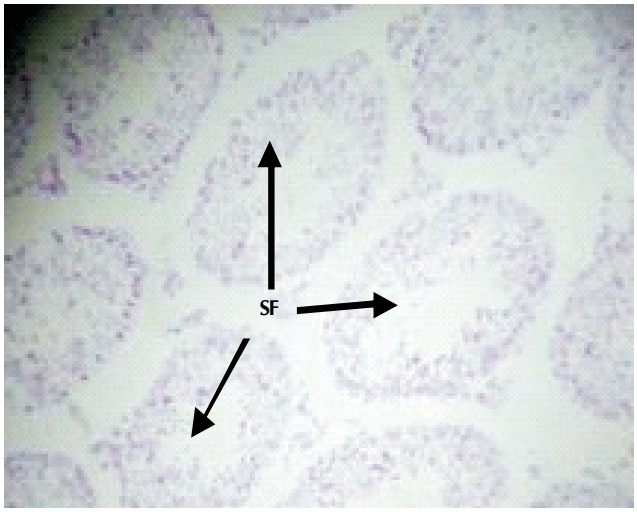

Figure 11: Section of testis of 96 hours post-treatment mice showing DNA content in the cells of semiferous tubules (SFs) after Feulgen's reacion. 100X

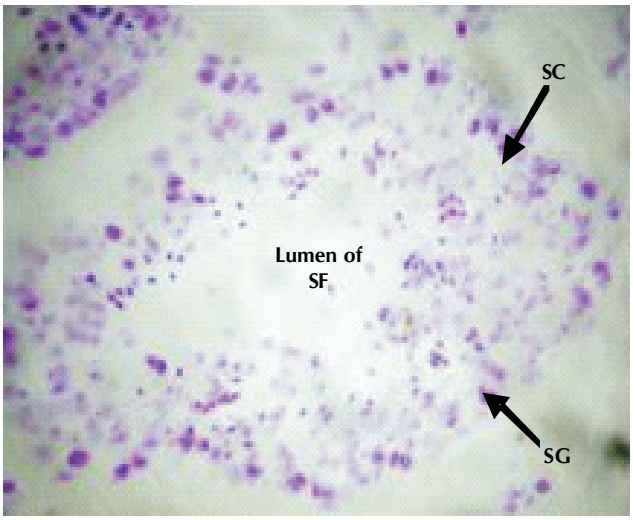

Figure 12: Section of testis of 96 hours post-treatment mice showing DNA content in the SGs, SCs, STs and in sperms after Feulgen's reacion. 400X

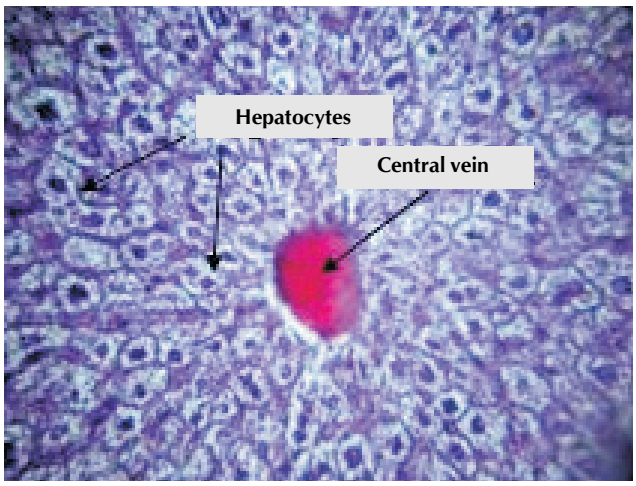

Figure 13: Section of lever of control mice showing normal histoarchitecture.

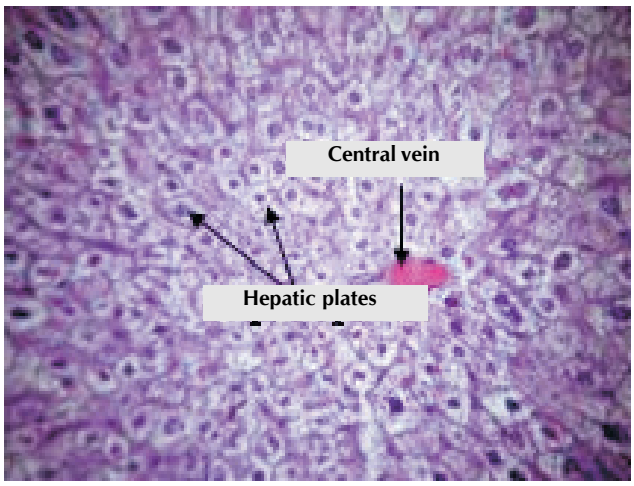

Figure 14: Section of liver of control mice showing central vein hepatocytes.

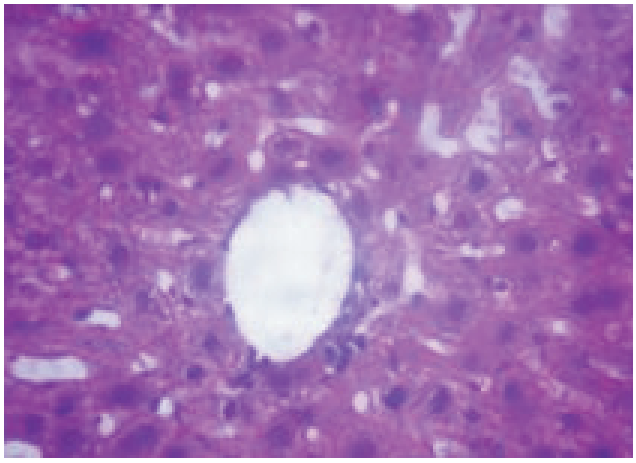

Figure 15: Section of 48 hours post-treatment liver of treated mice showing disintegration and cytoplasmic vacuolization's in cells.

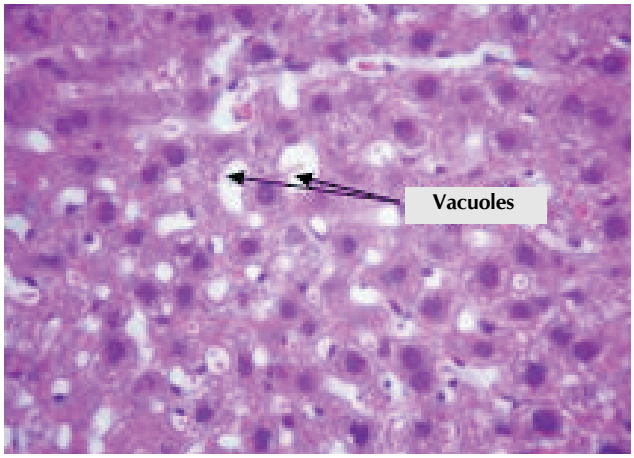

Figure 16: Section of 48 hours post-treatment liver of treated mice showing disruptions of normal hepatocyte's plates.

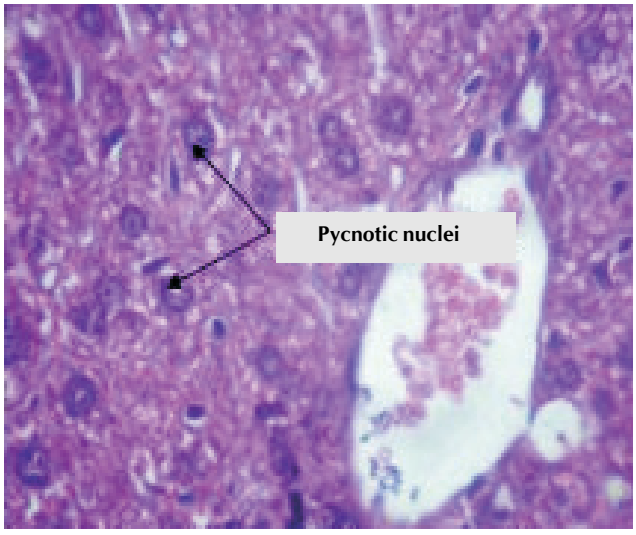

Figure 17: Section of 96 hours post-treatment liver of treated mice showing disintegration and cytoplasmic vacuolization in cells.

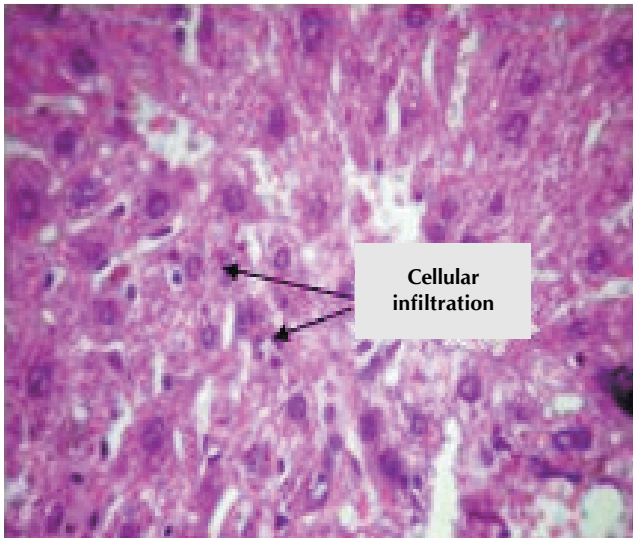

Figure 18: Section of 96 hours post-treatment liver of treated mice showing fibrosis and cellular infiltration and necrosis.

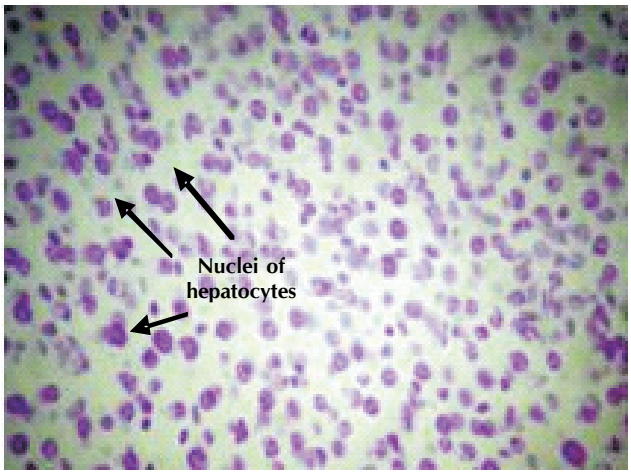

Figure 19: Section of liver of control mice showing DNA in nuclei of hepatoytes after Feulgen's reaction. 100X

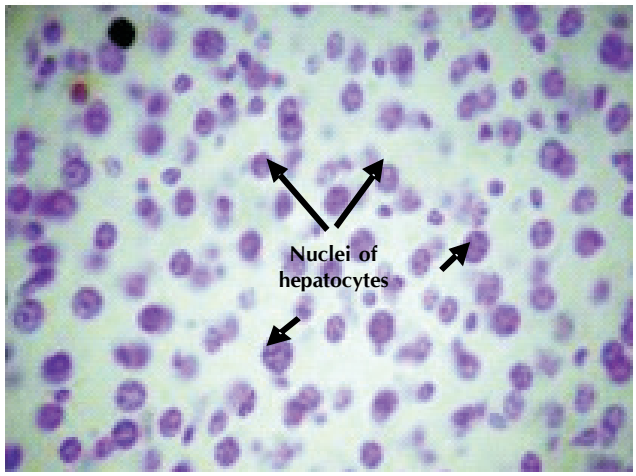

Figure 20: Section of liver of control mice showing DNA in nuclei of hepatocytes after Feulgen's reaction. 400X

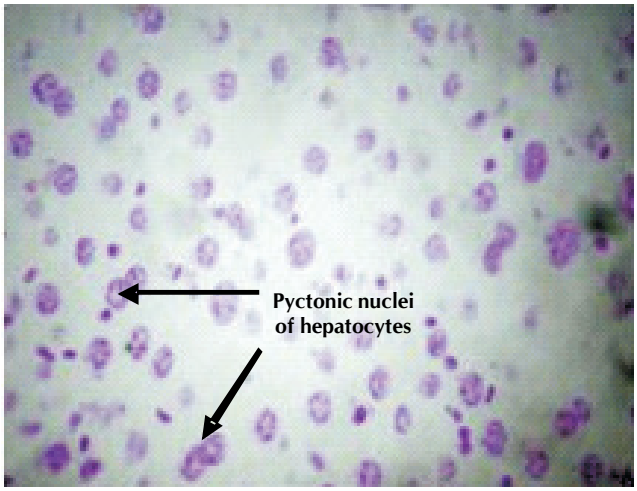

Figure 21: Section of liver of 48 hours post-treatment mice showing DNA in nuclei of hepatocytes after Feulgen's reaction. 400X

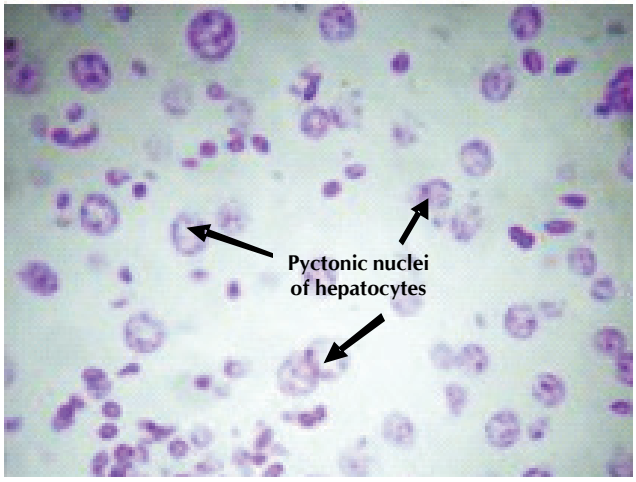

Figure 22: Section of liver of 48 hours post-treatment mice showing DNA in nuclei of hepatocytes after Feulgen's reaction. 400X

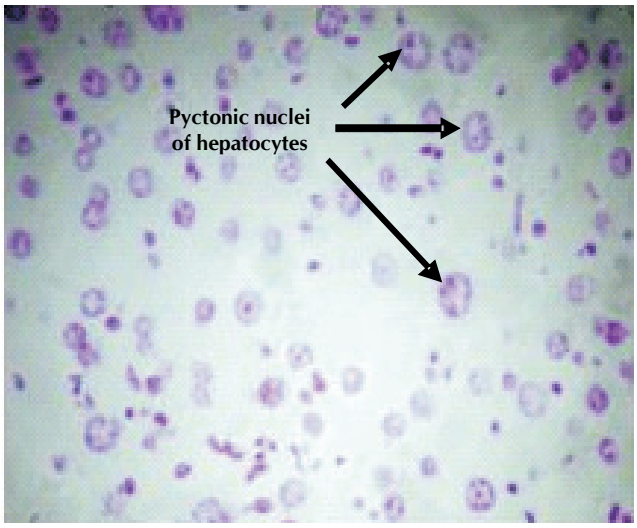

Figure 23: Section of liver of 96 hours post-treatment mice showing DNA in nuclei of hepatocytes after Feulgen's reaction. 400X

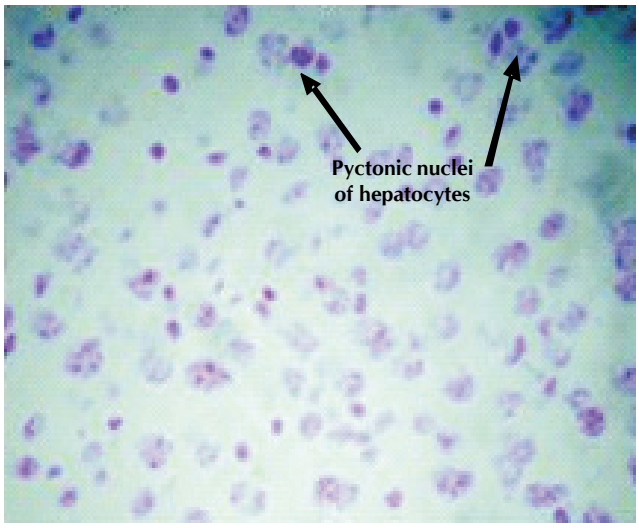

Figure 24: Section of liver of 96 hours post-treatment mice showing DNA in nuclei of hepatocytes after Feulgen's reaction. 400X

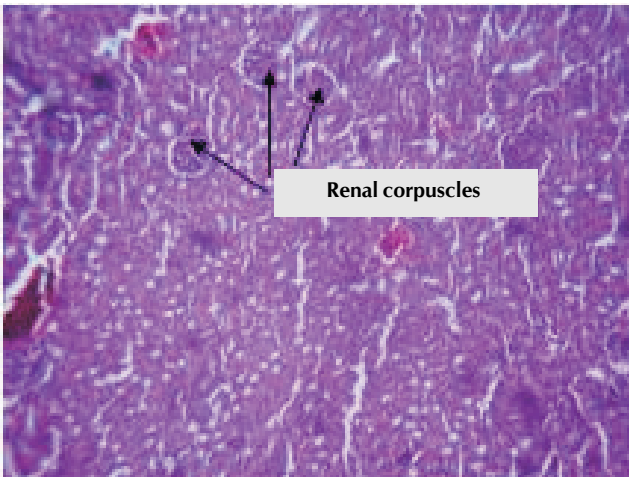

Figure 25: Section of kidney of control mice showing normal histoarchitecture. 100X

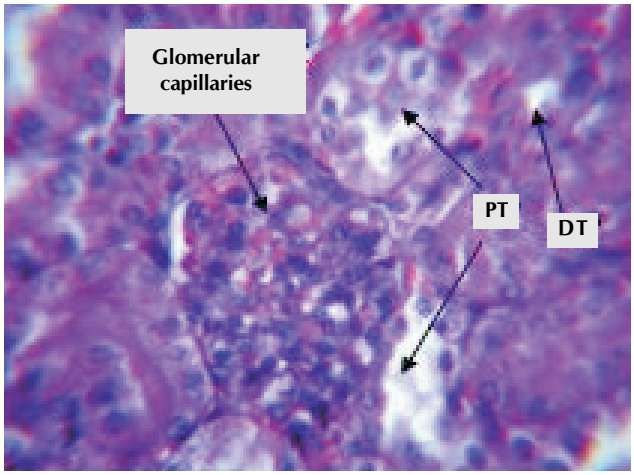

Figure 26: Section of kidney of control mice showing normal glomerulus and proximal tubule (PT) and distal tubule (DT). 400X

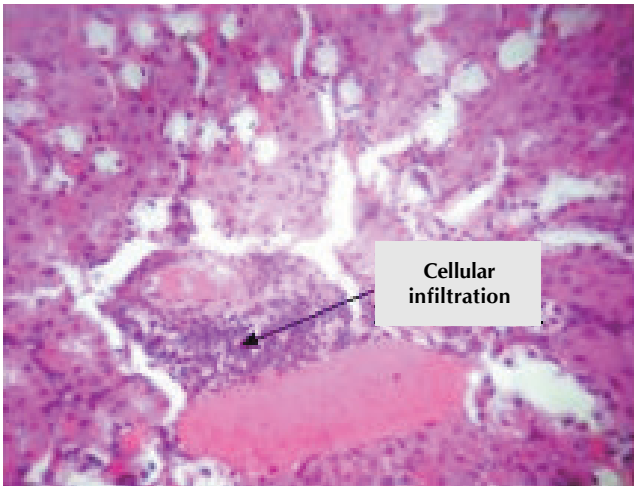

Figure 27: Section of 48 hours post-treatment kidney of treated mice showing disintegration of glomeruli and cellular infiltration. 400X

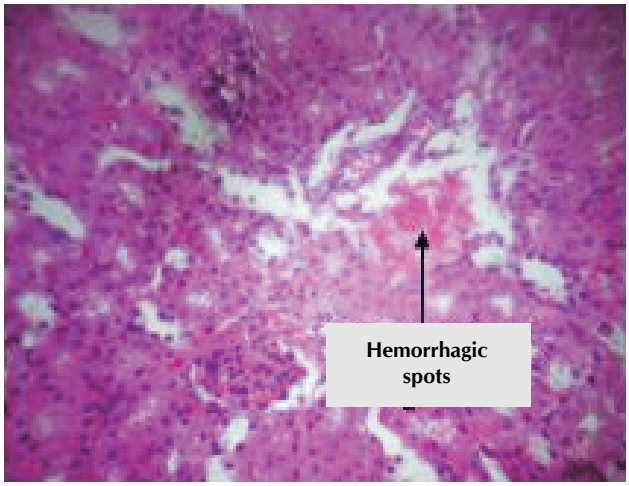

Figure 28: Section of 48 hours post-treatment of kidney of treated mice showing lesions of cortical tissues with hemorrhagic spots. 400X

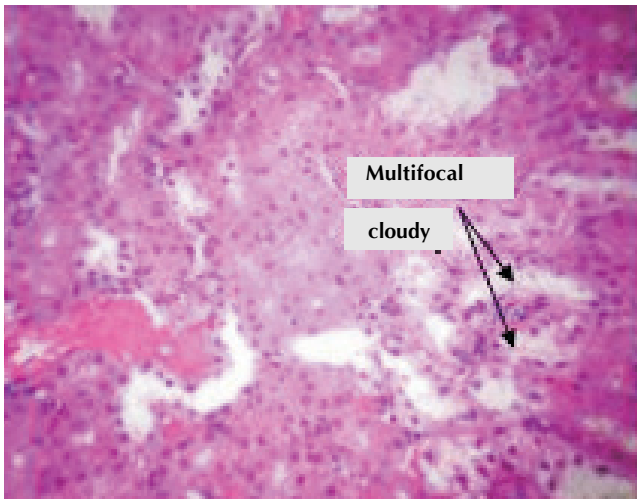

Figure 29: Section of 96 hours post-treatment kidney of treated mice showing multifocal cloudy, hydropic degeneration and cytoplasmic vacuolations of cells. 400X

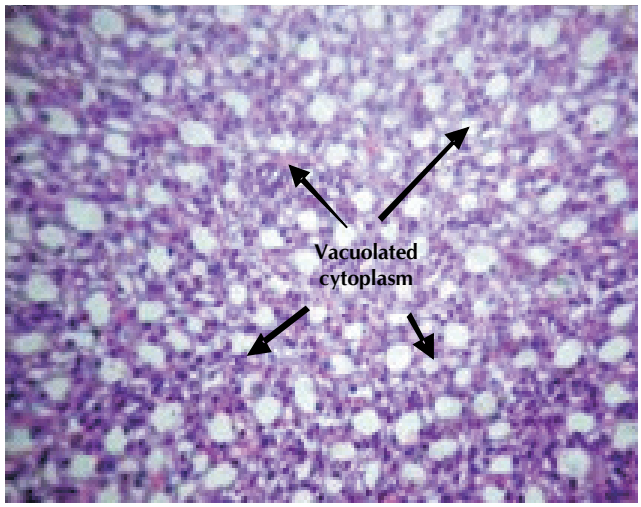

Figure 30: Section of 96 hours post-treatment of kidney of treated mice showing disintegration and cytoplasmic vacuolations of cells. 400X

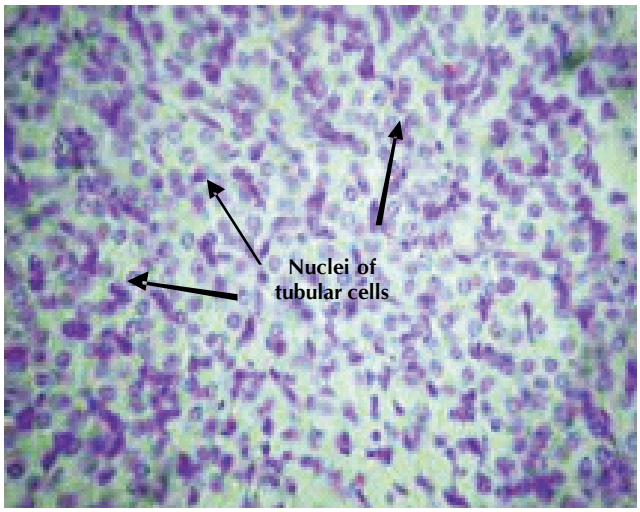

Figure 31: Section of kidney of control mice showing DNA in nuclei of tubular cells after Feulgen's reaction. 100X

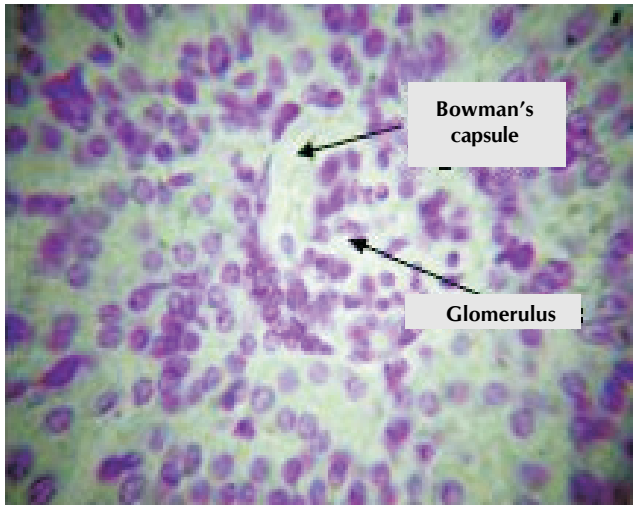

Figure 32: Section of kidney of control mice showing DNA in nuclei of tubular cells after Feulgen's reaction. 400X

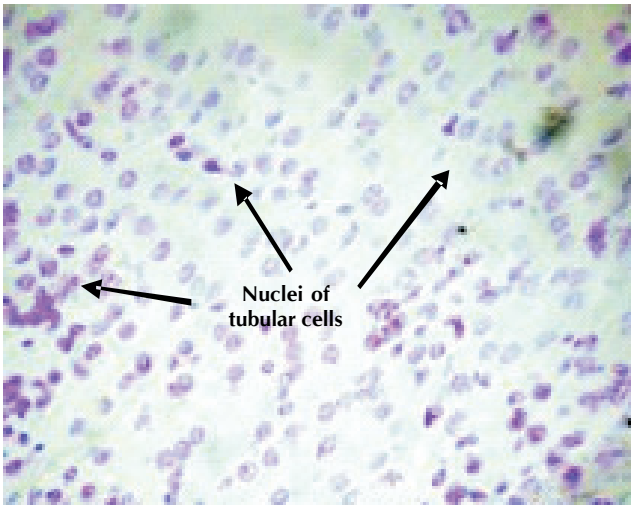

Figure 33: Section of kidney of 48 hours post-treatment mice showing DNA in nuclei of tubular cells after Feulgen's reaction. 100X

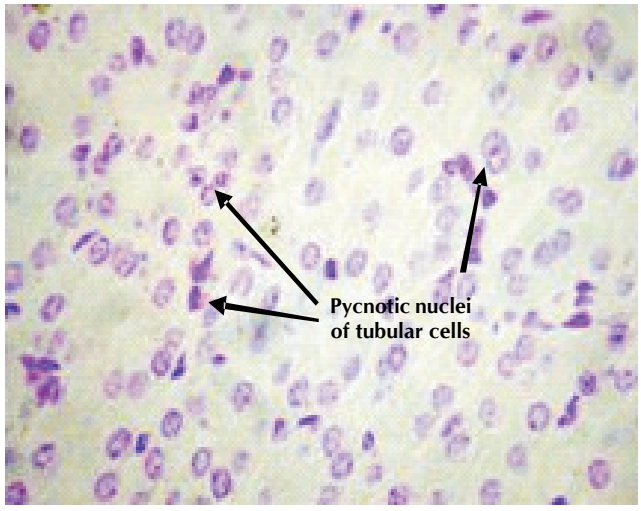

Figure 34: Selection of kidney of 48 hours post-treatment mice showing DNA in nuclei of tubular cells after Feulgen's reaction. 400X

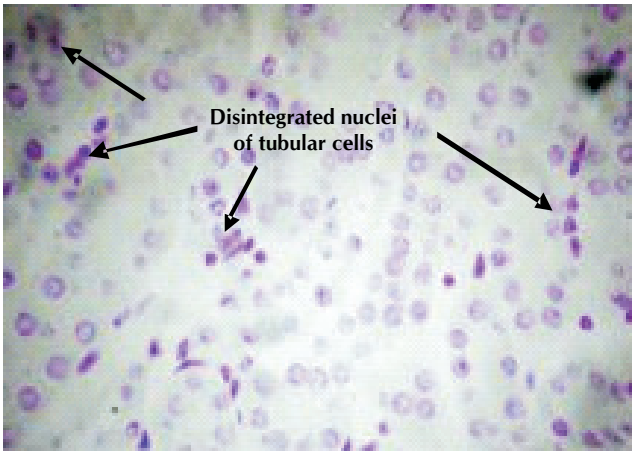

Figure 35: Section of kidney of 96 hours post-treatment mice showing DNA in nuclei of tubular cells after Feulgen's reaction. 100X

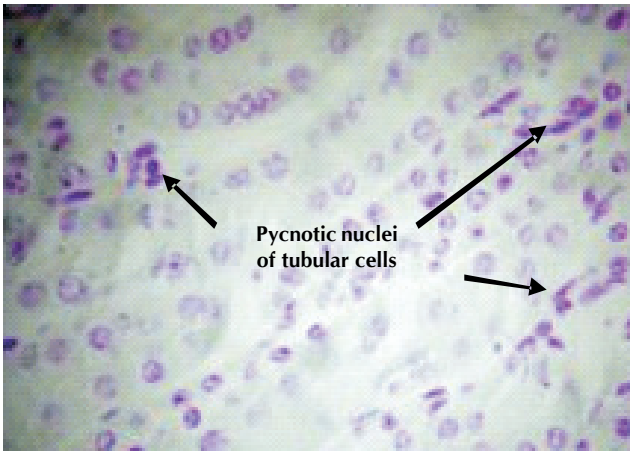

Figure 36: Selection of kidney of 96 hours post-treatment mice showing DNA in nuclei of tubular cells after Feulgen's reaction. 400X

However, there is scarce of studies regarding the genotoxic effect of heavy metals in animals. The results presented in this study clearly demonstrated that exposure of adult male mice to $CdCl_2$ induced genotoxic effect. Doreswamy *et al*. (2004) have shown that administration of heavy metal nickel induce DNA damage in testis of mice while Sharma *et al*. (1980) produced DNA damage in guinea pigs through cadmium chloride. In the present study, light pink staining of the nuclei of spermatogonia, spermatids and sperms in testes (Fig. 7-12); hepatocytes in liver (Fig. 19-24) and in the cells lining proximal convoluted tubules, Henle's loops, distal convoluted tubules and collecting ducts of kidney (Fig. 31-36) after Feulgen's reaction clearly indicated DNA damage by $CdCl_2$. Similar results have also been reported by Valverde *et al*. (2002) and Mohapatra *et al*. (2011) in mice on exposure to lead and arsenic respectively. Saleha Bano *et al*. (2001) clearly demonstrated genotoxic effect of arsenic trioxide in mice. In their study, a significant increase in comet tail length at all the doses clearly gives evidence that arsenic trioxide causes DNA damage effectively. The current study found that exposure to $CdCl_2$ resulted in significant histopathological abnormalities in testes. Distortion of the seminiferous tubules accompanied by disorganization of the seminiferous epithelium and cytoplasmic vacuolization, nuclear fragmentation and nuclear pycnosis in the spermatogonia, spermatocytes, spermatids and sertoli cells are more pronounced in 96h post-treatment mice. These results are in agreement with Saksena and Lau (1979) and Yang *et al*. (2006). Elbetieha and Al-Hamood (1997), Bataineh *et al*. (1998) and Mayyas *et al*. (2005) showed that the exposure of male rats to chromium chloride, potassium dichromate and aluminum chloride significantly reduced the weight of testes with necrotic changes. The current study also found that exposure to $CdCl_2$ resulted in a significant decrease in the sperm amount in seminiferous tubules. This reduction could be the result of reduced testicular function (reduced spermatogenesis) due to the internal damage suggested by the histological abnormalities such as cytoplasmic disintegration and nuclear pycnosis in sertoli and interstitial cells observed in the testes of the exposed mice. These findings are in agreement with several previous studies. Kwon *et al*. (1997), Bench *et al*. (1999) and Tbeileh *et al*. (2007) concluded that cadmium has a detrimental effect on testicular function that result in reduced sperm production. Fiorini *et al*. (2004) reported that testicular toxicant such as cadmium reduce or redistribute specific functional surface proteins on the sertoli cell membrane that are necessary for the development and maintenance of spermatogenesis.

The present study indicated that $CdCl_2$ induced marked histopathological alterations in the liver tissue of mice manifested by disruption of hepatocytic plates, disintegration of hepatocytes marked by rupture of cell membrane, cytoplasmic vacuolization and smaller sized nuclei showing pycnosis. Proposed mechanism for the cytoplasmic vacuolization has been given by Robins and Angell (1976) as one of the important responses to all forms of cell injury. The result of this study shows necrosis and disruption of hepatocytes which resulted in increased intercellular spaces leaving some empty spaces. Similar histopathological observations have been reported by Chishti and Rotkiewicz (1993) and Soni and Gupta (2006) in the liver on exposure to cadmium chloride and mercuric chloride. The current study showed widespread fibrosis, interstitial mononuclear cellular infiltration and hemorrhagic spots in liver of treated mice confirms the severity of the toxicity of heavy metal, cadmium.

The results of the current study indicate that oral administration of $CdCl_2$ induced lesions in the cortex and medulla with multifocal cloudy and hydropic degenerations with necrosis in the kidney. Similar pathological changes have earlier been reported in the kidneys of lower animals like fishes (Banerjee and Bhattacharya, 1994; Anitha Kumari and Sri Ram Kumar, 1997) and in cockerels (Chisti and Rotkiewicz, 1993) on exposure to various pollutants. Garba *et al*. (2007) noticed histopathological lesions which ranged from severe multifocal congestion, cystic dilation in the medulla, proteinaceous casts within ducts, interstitial mononuclear cellular infiltration with hemorrhage in the rat kidney. The results of the present study showed significant distortion of renal corpuscles, loss of cellular integrity of tubular cells with disrupted nuclei. These degenerative changes may be due to altered metabolic activity or due to metal ion-renal tissue interaction as suggested by Gupta and Rajbanshi (1979, 1982), Sharma and Sharma (1994) and Gupta and Srivastava (2006).

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