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Research Article
Structural Changes in Adult Rat Liver Following Cadmium Treatment

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Abstract

Background and Objective: Cadmium (Cd) is a natural heavy metal with no known positive biological function in humans. Therefore, it is not normally found in body fluids or tissues. However, its presence in the body of humans and animals can produce acute and chronic poisoning, leading to bony lesions, liver damage and much more. Biochemical, histopathological and histochemical liver changes due to injection of $CdCl_2$ in rats were examined as a model of chronic exposure and toxicity. In addition, possible recovery from the toxic effects of this heavy metal after withdrawal was also investigated. **Materials and Methods:** A total of 48 adult male rats albino were divided into two groups and subcutaneously injected every other day with equivalent volumes of saline solution (control group, n = 16) or $CdCl_2$ (experimental group, n = 32 total) for a total of 45 days. Experimental rats were further subdivided into subgroups A and B (n = 16 each). Rats in subgroup A were injected subcutaneously with 1/8 the median lethal dose of $CdCl_2$, while subgroup B was given a double dose of $CdCl_2$ (1/4 median lethal dose). On day 20, 30 and 45, 4 rats from each group were sacrificed and blood and liver samples were taken. The remaining 4 rats in each group were left for an additional 30 days without injection before blood and liver tissue were collected to evaluate possible signs of recovery. Functional, histological and histochemical examination of blood and liver tissue was conducted at each time-point. **Results:** Significant elevation of liver function was found in both experimental groups as indicated by increased serum alanine aminotransferase, aspartate aminotransferase and alkaline phosphatase levels. Histopathologically, liver samples showed many displaced lesions, ranging from hydropic degeneration to cellular necrosis and most of the portal vein radicals were dilated and congested. The extent of damage was more severe in rats treated for a longer period of time and a higher Cd dose. Mild recovery was observed in specimens examined 30 days after $CdCl_2$ withdrawal, especially in subgroup A. Histochemistry showed a gradual decrease in hepatocyte carbohydrate, DNA, RNA and total protein content over time, reaching a minimum by injection day 45 for subgroup B. While partial recovery of these factors was observed in hepatic cells 30 days after $CdCl_2$ withdrawal, especially in subgroup A, they never returned to control levels. **Conclusion:** The present study revealed that chronic subcutaneous exposure to $CdCl_2$ causes significant hepatotoxicity in less than a month. Therefore, workers dealing with Cd in industrial settings should use proper precautions and safety measures to avoid Cd exposure. Furthermore, cigarette smoke is known to contain Cd, antismoking programs should be implemented to reduce mortality from cardiovascular and respiratory diseases associated with Cd toxicity.

Key words: Cadmium toxicity, liver toxicity, cardiovascular diseases, respiratory diseases, biochemical changes

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Competing Interest: The authors have declared that no competing interest exists.

Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

Cadmium (Cd) is a heavy metal typically present in nature in combined form. Most of the Cd produced in the world is from smelting and refining of Zn, Pb and Cu ores. Industrially, Cd is used mainly for electroplating and Cd-bearing alloys[1]. The Cd can contaminate air, drinking water and food. Sources of Cd contamination in the air and water include industrial waste, insecticides and fertilizers, galvanized pipes that carry drinking water, cigarette smoke, coal, oil and wood consumption and smelting of rubber tires[2].

The Cd has no known beneficial biological function in humans or animals and is not normally found in body fluids or tissues. Its detection is therefore, reflective of environmental exposure[3]. On average, most foods contain less than 0.02 μg Cd g^{-1} and human dietary intake ranges from 0.2-0.7 μg kg^{-1} body weight for an adult and exceeds 1 μg kg^{-1} body weight in younger age groups[4]. Recently, a survey of Cd contamination in boiled liver, lung and kidney products within Japanese markets found that Cd levels were 8.59±1.20, 10.4±.86 and 1.66±.13 μg/wet tissue g, respectively[5]. Moreover, studies have shown that one cigarette contains about 1.7 μg of Cd. Thus, smokers who inhale 20 cigarettes/day can accumulate as much as 0.5 mg of Cd in their tissues in just 1 year[6].

The Cd has a very long biological half-life in the human body (10-40 years), especially in the kidneys. However, the acute toxic effects of Cd are generally limited to the lungs and gastrointestinal tract. Chronic Cd exposure in humans has multiple effects, including nephropathy, bone lesions, pulmonary emphysema and cardiovascular manifestations. Once in the blood stream, Cd is deposited in different tissues to varying degrees, with the greatest concentrations in the liver and kidney[7].

Other studies in animals show cadmium toxicity lead to increase plasma uric acid and nephropathy as a result of this accumulations[8] and proven environmental pollutant with hepatotoxic effects[9] that include a rise in hepatic enzymes, serum alanine aminotransferase and γ-glutamyltranspeptidase. Furthermore, there is evidence supporting the carcinogenicity of Cd, which has resulted in its being classified as a probable carcinogen[10]. Other studies refer this toxicity to increase oxidative stress[11] or response[12-14]. The present study aimed to detect the biochemical, histopathological and histochemical changes that occur in the liver of rats following chronic administration of $CdCl_2$. In addition, possible recovery from the possible toxic effects of this heavy metal after withdrawal was also evaluated.

MATERIALS AND METHODS

The $CdCl_2$ obtained from Fluka Chemika, New York, USA (molecular weight = 219.34 g moL^{-1}, specific gravity = 4.047 g cm^{-3} at 25°C) was soluble in water and alcohol. According to Reed and Munech[10] the median lethal dose of $CdCl_2$ for adult rats was found to be approximately equal to 20 mg kg^{-1} body weight.

Experimental rats: A total of 48 adult male albino rats (150-250 g body weight) were included in the present study, rats were fed on basal ration composed of wheat bran, soya bean powder 44%, fish meal, molasses, fibers 3.3%, sodium chloride, calcium carbonate, calcium phosphate, methionine and ash with net protein 22% and fats 4.7%. Rats were divided into two main groups and subcutaneously injected with equivalent volumes of either saline (NaCl) solution (controls, n = 16) or $CdCl_2$ (experimental group, n = 32 total) every other day for a total of 45 days. The experimental group was further subdivided into subgroups A and B (n = 16 rats each, Table 1).

Rats in subgroup A were injected with 1/8 LD_{50} of $CdCl_2$ (2.5 mg $CdCl_2$ kg^{-1} body weight), while subgroup B rats were given a double dose of $CdCl_2$ (1/4 LD_{50} = 5 mg kg^{-1} body weight).

On injection days 20, 30 and 45 of the study period, 4 rats from each of the 3 groups (control, subgroup A and subgroup B) were sacrificed in order to collect blood (heart puncture) and liver tissue samples. The remaining 4 rats in each group were left for an additional 30 days without injection before being sacrificed to collect blood and tissue.

Table 1: Number of rats sacrificed at each time point

	Groups			
		$CdCl_2$ treated		
Number	Control	Subgroup A (2.5 $CdCl_2$/kg body weight)	Subgroup B (5 $CdCl_2$/kg body weight)	Injected period (day)
12	4	4	4	20
12	4	4	4	30
12	4	4	4	45
12	4	4	4	After 30 days withdrawal
48	16	16	16	

These rats (n = 12 total) were used to examine any signs of recovery which might have occurred due to $CdCl_2$ withdrawal. All rats were euthanized and samples taken immediately after sacrifice. Functional, histological and histochemical tests were conducted on hepatic cells in blood and in liver tissue.

Histopathology and histochemistry: Following dissection, small pieces of the liver were taken and immediately placed in the proper fixative. For histopathological examination, specimens were fixed in 10% neutral formalin and prepared for haematoxylin and eosin staining[15]. For histochemical studies, some specimens were fixed in alcoholic Bouin's solution to observe carbohydrate content using the Hotchkiss[16] periodic acid-Schiff (PAS) technique. Other specimens were fixed in Carnoy's solution for observation of total proteins, DNA and RNA. DNA was visualized using Feulgen's technique[17], where in the Schiff reagent reacts with exposed aldehyde groups released by hydrolysis of the deoxypentose sugars of the DNA to produce a reddish-purple color in the nuclear chromatin. The RNA was revealed in paraffinized tissue sections treated with methyl green-pyronin[18]. Nucleolar and cytoplasmic RNA stained pink to reddish indicates the presence of RNA, whereas, structures containing DNA stain bluish-green. Total protein in paraffinized sections fixed in Carnoy's solution were stained a bluish color using 0.1% alcoholic bromophenol blue saturated with mercuric chloride[19].

RESULTS AND DISCUSSION

Biochemical changes: The results showed highly significant elevations in the serum levels of alanine aminotransferase, aspartate aminotransferase and alkaline phosphatase in the experimental group, especially subgroup B, as shown in Table 2.

Tissue observations: The liver of experimental versus control rats appeared enlarged, pale brown, soft in consistency. These changes became gradually more frequent in occurrence and pronounced in rats treated for longer periods of time, as well as for those given a higher $CdCl_2$ dose.

Histopathology

Subgroup A (1/8 LD_{50} $CdCl_2$): Subgroup A liver specimens examined on injection day 20 showed various degenerative changes compared to the control group (Fig. 1, 2). The central vein showed dilatation and hydropic degeneration characterized by marked loss of uniformity and regularity of the hepatic lobules was observed (Fig. 3). A large number of inflammatory cells were observed infiltrating the portal tract together with congestion and dilatation of the portal vein radical, as well as sinusoidal obliteration. Moreover, some of the nuclei became pyknotic (Fig. 4).

Specimens taken on injection day 30 compared to control showed more cellular degeneration (Fig. 5, 6). Marked cytoplasmic granularity and hydropic degeneration were also seen. Many nuclei showed karyorrhexis or complete disappearance, indicating an advanced degree of karyolysis. There was also marked nuclear pyknosis and an increase in the number of von Kupffer cells (VKCs). In addition, the central vein showed moderate congestion and dilatation. Blocking of the portal tract with a large number of mononuclear inflammatory cells and congestion of the portal vein radical was also observed (Fig. 6).

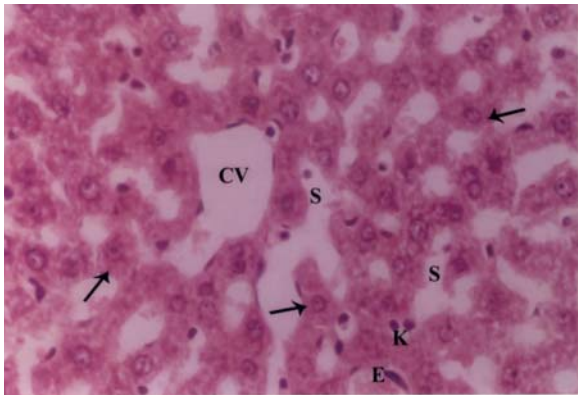

Fig. 1: Photomicrograph of a control liver section showing normal structural morphology in the central area, sinusoids (S) and central vein (CN), as well as normal hepatocyte (arrows), von Kupffer KJ and endothelial (E) cell morphologies. Haematoxylin and eosin staining, 400X objective

Table 2: Liver function tests after 45 days of treatment injection

	Control group	Subgroup A (1/8 median lethal dose)	Subgroup B (1/4 median lethal dose)
ALT (U L^{-1})	25.31±2.52	175.63±9.33***	191.22±12.41***
AST (U L^{-1})	36.13±2.40	311.25±13.71***	392.65±16.35***
Alkaline Phosphatase (u/100 mL)	9.11±0.57	50.27±4.55***	61.52±3.81**

Data are expressed as the Mean±standard deviation, ***p<0.005, LD_{50}, median lethal dose, ALT: Alanine aminotransferase, AST: Aspartate aminotransferase

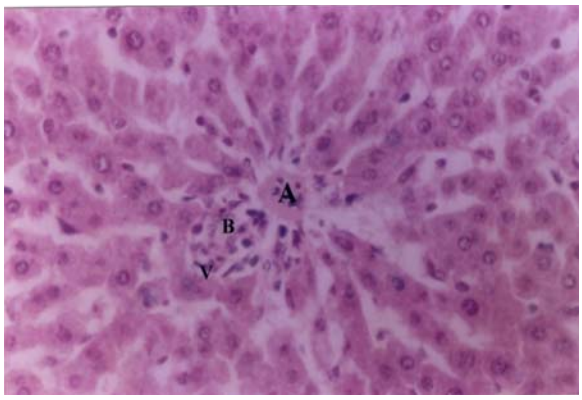

Fig. 2: Photomicrograph of a control liver section showing the normal portal area, including the bile ductule (B), a portal vein radical (V) and hepatic artery (A). Haematoxylin and eosin staining, 400X objective

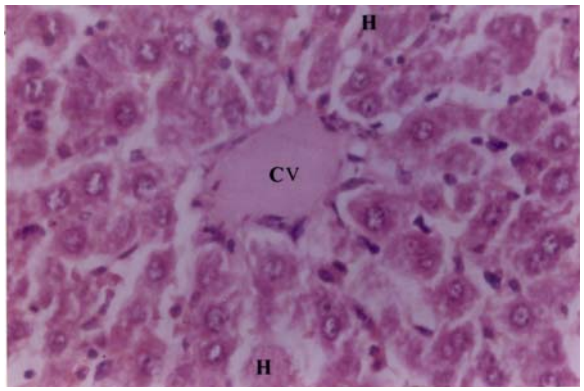

Fig. 3: Photomicrograph of a subgroup A (1/8 LD_{50} $CdCl_2$) liver section on injection day 20 showing mild hydropic degeneration (H) and dilatation of the central vein (CV). Haematoxylin and eosin staining, 400X objective

Materials examined on injection day 45 compared to control showed massive degenerative changes manifested by marked hydropic degeneration, cytoplasmic granularity and vacuolation. Severe nuclear changes in the form of karyolysis and single cell necrosis were noted. There was also a marked increase in pyknotic nuclei, especially in the peripheral hepatocytes and VKC hyperplasia (Fig. 7). Portal areas showed a severe increase in the number of mononuclear inflammatory cells invading all sections of the portal tract and between hepatocytes (Fig. 8).

Partial recovery involving incomplete restoration of the liver structure was achieved in subgroup A after the 30 days withdrawal period. The regenerative signs were manifested by a pronounced increase in the number of binucleated cells and an increase in phagocytic VKC hyperplasia, which engulfed

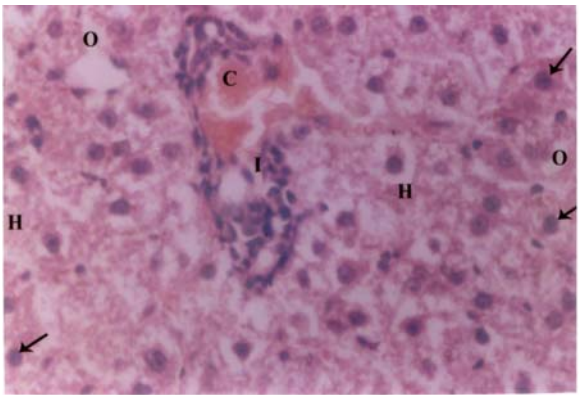

Fig. 4: Photomicrograph of a subgroup A (1/8 LD_{50} CdCl2) liver section on injection day 20 showing congestion and dilatation of the portal vein radical (C), hydropic degeneration (H), nuclear pyknosis (arrows), sinusoidal obliteration (O) and infiltration of the portal tract with inflammatory cells (I). Haematoxylin and eosin staining, 400X objective

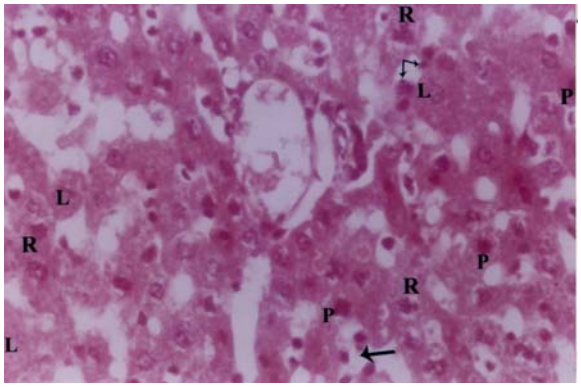

Fig. 5: Photomicrograph of a subgroup A (1/8 LD_{50} $CdCl_2$) liver section on injection day 30 showing karyorrhexis (R), karyolysis (L) and nuclear pyknosis (P), with a marked increase in the number of von Kupffer cells (arrows). Haematoxylin and eosin staining, 400X objective

and eliminated the necrotic debris of the liver (Fig. 9). However, the degenerative changes were still apparent and disturbed the normal configuration of the lobules in some areas of the hepatic tissue. Pyknosis, karyolysis and single cell hepatocyte necrosis was still markedly apparent.

Subgroup B (1/4 LD_{50} $CdCl_2$): The histopathological changes in the liver of subgroup B rats on injection day 20 were significantly different compared to subgroup A. Portal tracts were blocked by enormous numbers of inflammatory cells, with congestion of portal vein radicals (Fig. 10). Pyknosis and

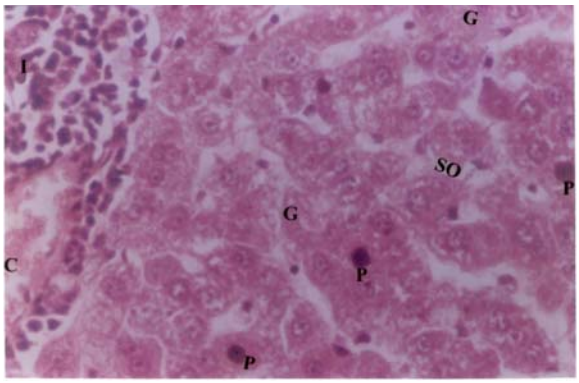

Fig. 6: Photomicrograph of a subgroup A (1/8 LD_{50} $CdCl_2$) liver section on injection day 30 showing blocking of the portal tract with a large number of inflammatory cells (I) and congestion (C) of the portal vein radical. Nuclear pyknosis (P), cytoplasmic granularity (G) and sinusoidal obliteration (SO) are also shown. Haematoxylin and eosin staining, 400X objective

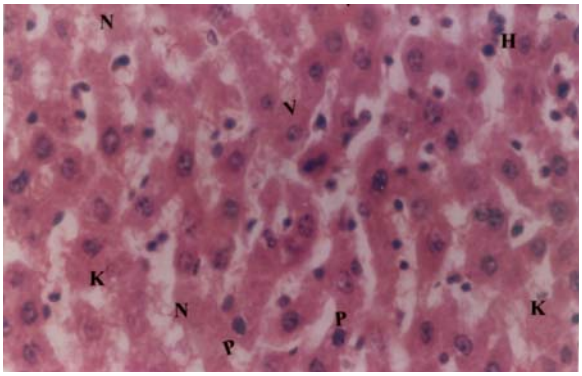

Fig. 7: Photomicrograph of a subgroup A (1/8 LD_{50} $CdCl_2$) liver section on injection day 45 showing pyknotic nuclei (P), hyperplasia of Von Kupffer cells (H), karyolysis (K), single cell necrosis (N) and severe hydropic degeneration with vacuolation (V). Haematoxylin and eosin staining, 400X objective

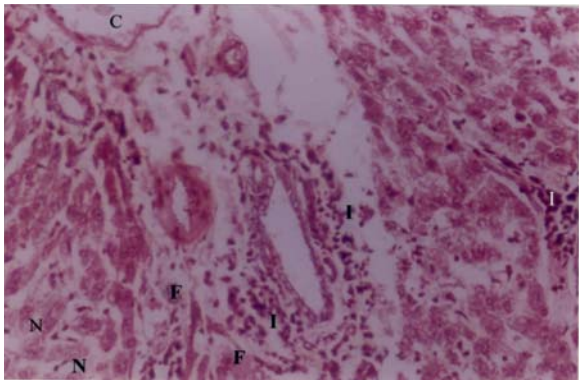

Fig. 8: Photomicrograph of a subgroup A (1/8 LD_{50} $CdCl_2$) liver section on injection day 45 showing marked dilatation of the portal vein radical (C), severe infiltration of portal tract areas with inflammatory cells (I), single cell necrosis (N) and mild fibrosis (F). Haematoxylin and eosin staining, 250X objective

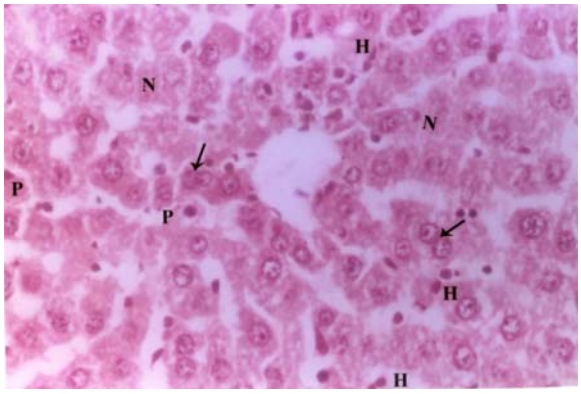

Fig. 9: Photomicrograph of a subgroup A (1/8 LD_{50} $CdCl_2$) liver section on injection day 45 and 30 days after $CdCl_2$ withdrawal showing a large amount of Von Kupffer cell hyperplasia (H), pyknotic nuclei (P), necrotic cells (N) and many binucleated cells (arrows). Haematoxylin and eosin staining, 400X objective

karyolysis of different hepatic nuclei are obvious (Fig. 11) and there was marked dilatation of the central vein, which also contained inflammatory and red blood cells. On injection day 30, sections showed massive deterioration of hepatic tissues. Many cells appeared necrotized, as either discrete or aggregated focal areas of necrosis and there was infiltration of the portal tract radicals with inflammatory cells (Fig. 12).

Specimens examined on injection day 45 showed intensive parenchymal degeneration with a large amount of hepatic cell necrosis. Focal necrotic areas were clearly observed in this group and were greatest relative to all other treatment groups and days. Coagulative necrosis was accompanied with marked liver fibrosis and inflammatory cells which replaced most of the hepatic cells. In the peripheral zones, some pyknotic nuclei were observed (Fig. 13). Intensive dilatation and congestion of the central vein, which was filled with red blood cells, was also observed. In addition, it is observed edema in the wall of the central vein with a massive increase in the number of inflammatory cells scattered around the central vein denoting intensive inflammation (Fig. 14).

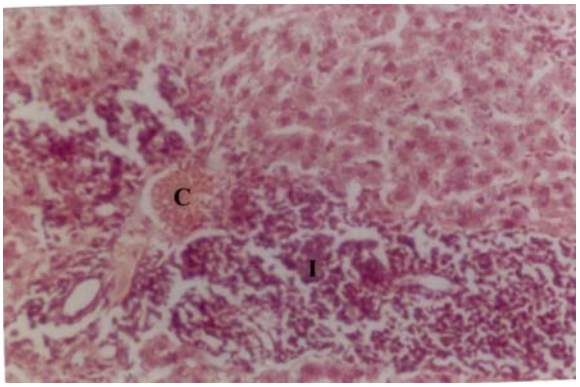

Fig. 10: Photomicrograph of a subgroup B (1/4 LD_{50} $CdCl_2$) liver section on injection day 20 showing blockage of the portal tract with an enormous number of inflammatory cells (I) and congestion (C) of the portal vein radical. He Haematoxylin matoxylin and eosin staining, 250X objective

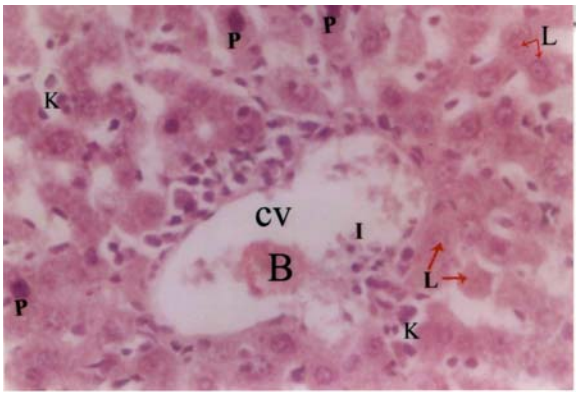

Fig. 11: Photomicrograph of a subgroup B (1/4 LD_{50} $CdCl_2$) liver section on injection day 20 showing an obvious increase in the number of pyknotic nuclei (P) and Von Kupffer cells (K), in addition to karyolysis (L). The central vein (CV) is markedly dilated and contains inflammatory (I) and red blood cells. Haematoxylin and eosin staining, 400X objective

In contrast to subgroup A, subgroup B specimens examined 30 days after $CdCl_2$ withdrawal showed very little restoration of normal liver architecture. The regenerative signs that could be observed were manifested by a pronounced increase in the number of the phagocytic VKCs, which remove necrotic debris in hepatic tissue. Nonetheless, significant degenerative changes were still observed in a large number of parenchymal cells, such as pyknosis and single cell necrosis (Fig. 15).

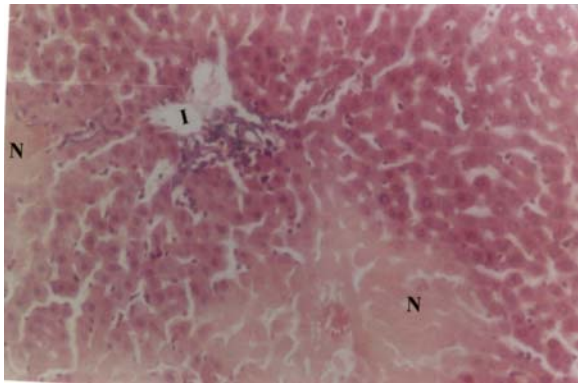

Fig. 12: Photomicrograph of a subgroup B (1/4 LD_{50} $CdCl_2$) liver section on injection day 30 showing large areas of focal necrosis (N) and infiltration of the portal tract radical with inflammatory cells (I). Haematoxylin and eosin staining, 250X objective

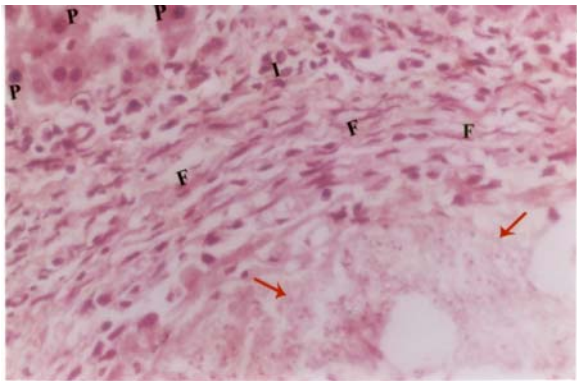

Fig. 13: Photomicrograph of a subgroup B (1/4 LD_{50} $CdCl_2$) liver section on injection day 45 showing marked hepatic fibrosis (F) reacting with inflammatory cells (I) and coagulation necrosis (arrows). Peripheral pyknotic nuclei (p) are also present. Haematoxylin and eosin staining, 400X objective

Histochemistry

Carbohydrates: Compared to the control group (Fig. 16), chronic administration of 1/8 LD_{50} $CdCl_2$ (subgroup A) for 45 days induced maximum depletion of glycogen content in rat hepatocytes. A large number of hepatic cells were PAS-negative, while other cells had a few scattered granules in their cytoplasm (Fig. 17). Moderate restoration of glycogen content was noted in specimens examined 30 days after 1/8 LD_{50} $CdCl_2$ withdrawal (Fig. 18). In subgroup B rats (1/4 LD_{50} $CdCl_2$), glycogen particles were completely absent in most of the cells (Fig. 19). Thirty days after $CdCl_2$ withdrawal,

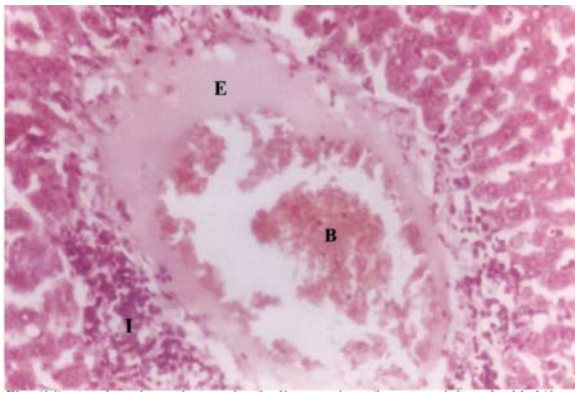

Fig. 14: Photomicrograph of a subgroup B (1/4 LD_{50} $CdCl_2$) liver section on injection day 45 showing marked dilatation and congestion of the central vein, which is filled with red blood cells (B), as well as edema (E) in the wall of the central vein. The central vein is surrounded by many inflammatory cells (I). Hematoxylin and eosin staining, 400X objective

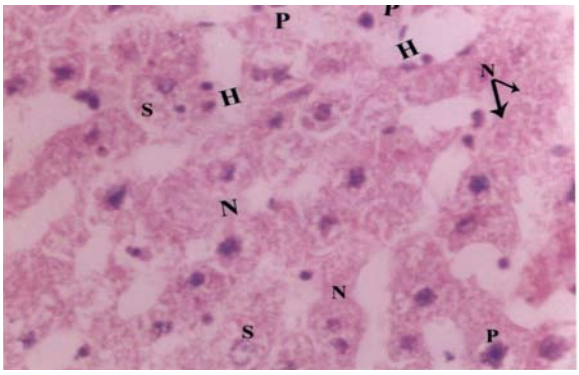

Fig. 15: Photomicrograph of a subgroup B (1/4 LD_{50} $CdCl_2$) liver section on injection day 45 and 30 days after $CdCl_2$ withdrawal showing very little recovery of hepatic tissue and an increase in the number of Von Kupffer cells (H). A large number of necrotic cells (N), karyolysis (S) and pyknotic nuclei (P) are still present. Hematoxylin and eosin staining, 400X objective

slight restoration of carbohydrate inclusions was noted in some hepatocytes, other cells were still PAS-negative (Fig. 20).

DNA: Compared with controls (Fig. 21), subgroup B specimens examined after injection day 45 showed maximum depletion of chromatin bodies in most hepatic cell nuclei. Many nuclei were shrunken and densely stained, while others were enlarged and showed migration of chromatin bodies along the nuclear membrane and had prominent nucleoli (Fig. 22).

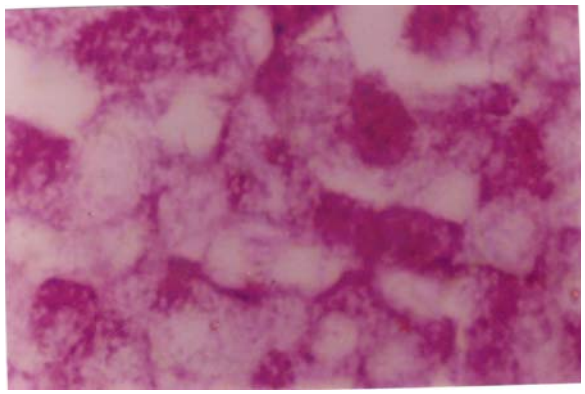

Fig. 16: Photomicrograph of a control liver section showing PAS-positive glycogen inclusions (deep purple, coarse particles) of different sizes densely located in the cytoplasm. The nuclei, however, are PAS-negative. 1000X objective

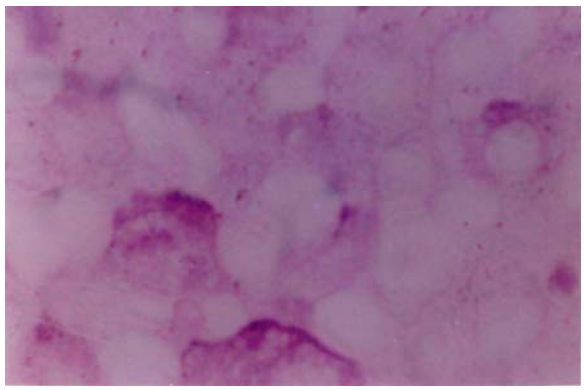

Fig. 17: Photomicrograph of a subgroup A (1/8 LD_{50} $CdCl_2$) liver section on injection day 45 showing marked glycogen depletion in the majority of hepatocytes. PAS staining, 1000X objective

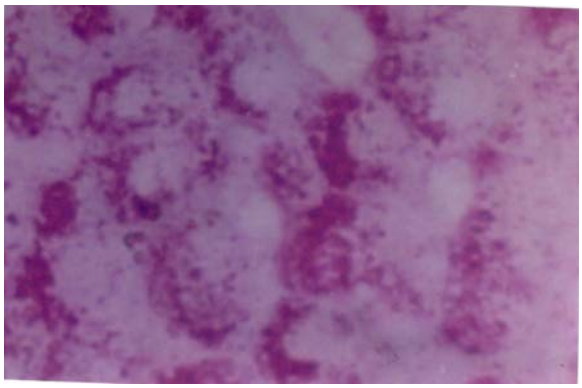

Fig. 18: Photomicrograph of a subgroup A (1/8 LD_{50} $CdCl_2$) liver section on injection day 45 and 30 days after $CdCl_2$ withdrawal showing moderate recovery of glycogen inclusions. PAS staining, 1000X objective

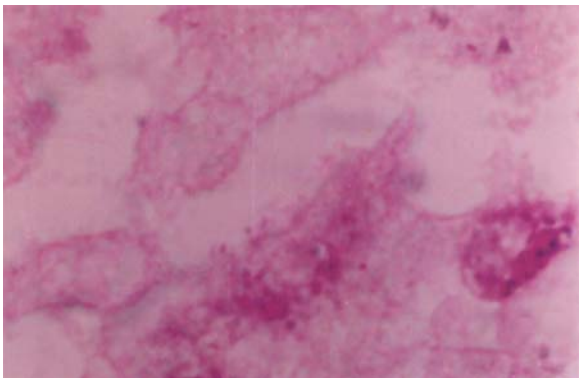

Fig. 19: Photomicrograph of a subgroup B (1/4 LD_{50} $CdCl_2$) liver section on injection day 45 showing complete absence of glycogen particles in most hepatocytes. Mild staining can be seen in a few cells. PAS staining, 1000X objective

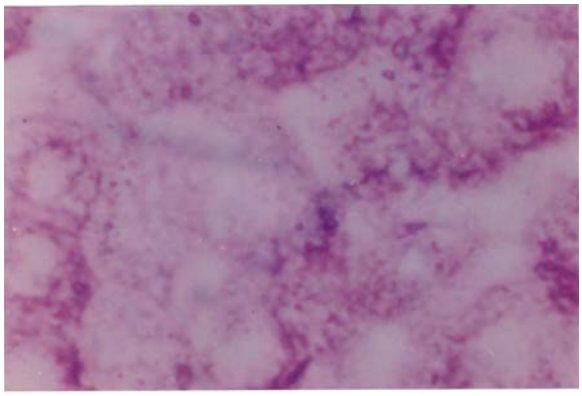

Fig. 20: Photomicrograph of a subgroup B (1/4 LD_{50} $CdCl_2$) liver section on injection day 45 and 30 days after $CdCl_2$ withdrawal showing slight restoration of the carbohydrate material in some hepatocytes. PAS staining, 1000X objective

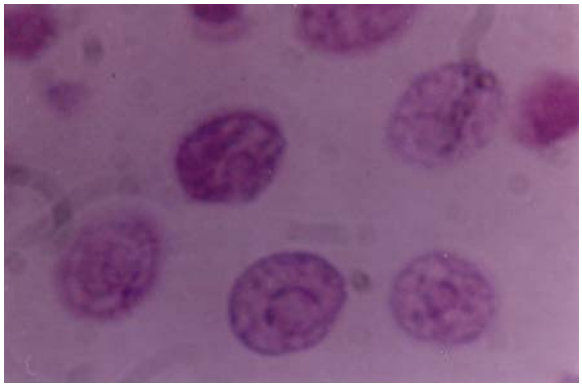

Fig. 21: Photomicrograph of a control liver section showing chromatin bodies as red-stained granules of DNA. Feulgen technique, 1000X objective

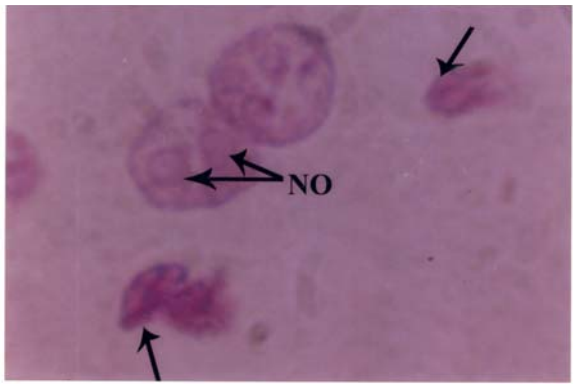

Fig. 22: Photomicrograph of a subgroup B (1/4 LD_{50} $CdCl_2$) liver section on injection day 45 showing severe depletion of chromatic bodies. Many nuclei are shrunken and densely stained (arrows), while others are enlarged and show chromatin migration along the nuclear membrane, with prominent nucleoli (NO). Feulgen technique, 1000X objective

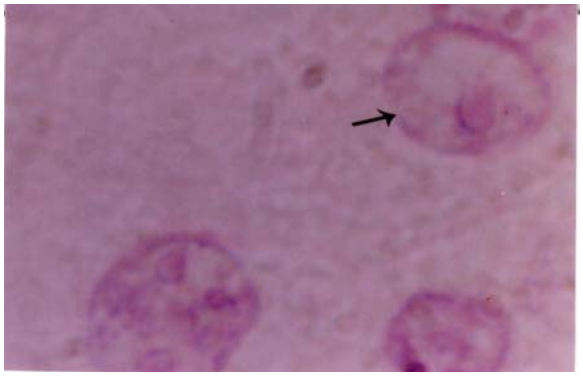

Fig. 23: Photomicrograph of a subgroup B (1/4 LD_{50} $CdCl_2$) liver section on injection day 45 and 30 days after $CdCl_2$ withdrawal showing mild restoration of DNA content. Some nuclei are still faintly stained (arrow). Feulgen technique, 1000X objective

However, mild restoration of DNA inclusions was detected in specimens examined 30 days following 1/4 LD_{50} $CdCl_2$ withdrawal (Fig. 23). At this time point, we also observed slight Feulgen reactivity, while some nuclei were still faintly stained due to marked depiction of chromatin particles.

RNA: The marked loss of pyronin-reactive RNA inclusions was observed after 45 days of chronic 1/8 LD_{50} $CdCl_2$ application in most hepatic cells (Fig. 25) compared with the control group, which showed RNA inclusions scattered randomly in the cytoplasm of hepatocytes. Although the nuclei of hepatic cells stained positive (greenish-blue) for DNA (Fig. 24), the intensity was weak, indicating their DNA contents were negatively

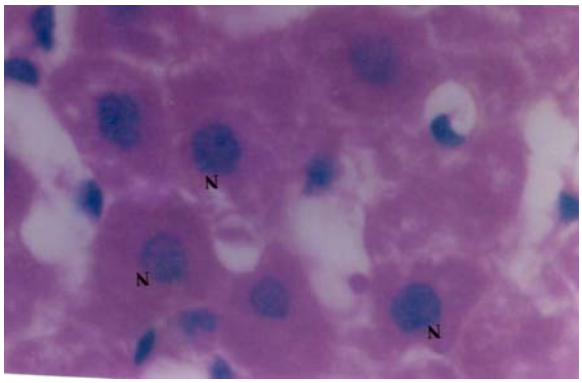

Fig. 24: Photomicrograph of a control liver section showing RNA content as small patches scattered randomly in the cytoplasm. The nuclei (N) are positively stained (greenish-blue) indicating their DNA content. Methyl green-pyronin staining, 1000X objective

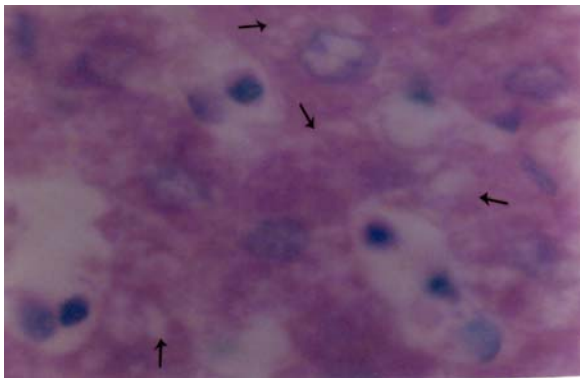

Fig. 25: Photomicrograph of a subgroup A (1/8 LD_{50} $CdCl_2$) liver section on injection day 45 showing marked loss of RNA inclusions (arrows), which are weakly stained with pyronin in most hepatocytes. Weak nuclear staining indicates marked DNA loss. Methyl green-pyronin staining, 1000X objective

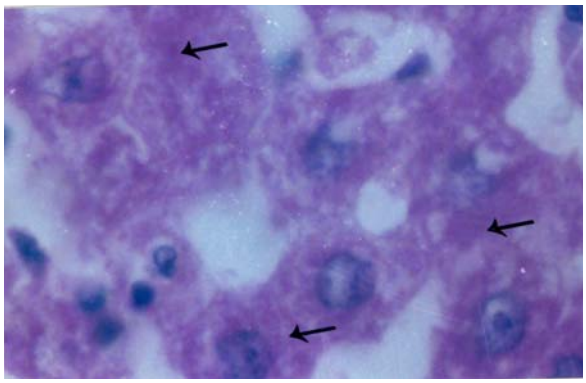

Fig. 26: Photomicrograph of a subgroup A (1/8 LD_{50} $CdCl_2$) liver section on injection day 45 and 30 days after $CdCl_2$ withdrawal showing partial restoration of RNA content (arrows). Methyl green-pyronin staining, 1000X objective

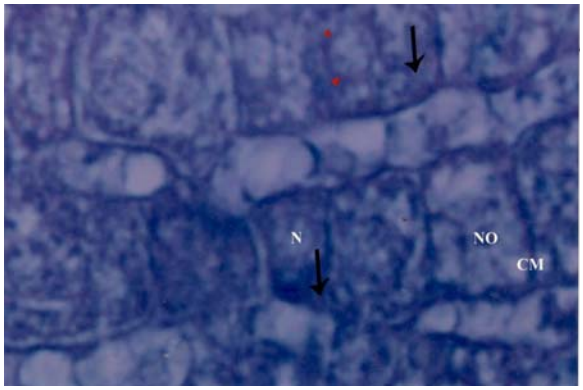

Fig. 27: Photomicrograph of a control liver section showing protein granules scattered randomly in the cytoplasm (arrows). The nuclei (N), nucleoli (NO), nuclear membrane (arrow head) and cellular membrane (CM) are intensely stained. Bromophenol blue staining, 1000X objective

affected by the presence of Cd (Fig. 25). On the other hand, partial restoration of RNA inclusions was observed in specimens 30 days after 1// LD_{50} $CdCl_2$ withdrawal (Fig. 26), which revealed mild pyronin reactivity in the cytoplasm of most hepatic cells.

Total protein: Compared to controls (Fig. 27), there was marked loss of protein inclusions in subgroup B specimens on injection day 45. Marked loss of total protein was observed in hepatic cells. Additionally, the cytoplasm and nuclei of many of these cells showed weak bromophenol blue staining, indicating marked destructive changes in the hepatic tissue (Fig. 28). The protein content in most hepatocytes was limited to the narrow cytoplasmic areas lying between unstained vacuoles. After 30 days of $CdCl_2$ withdrawal, slight restoration of total protein inclusions in the cytoplasm and nuclei were observed (Fig. 29).

The increasing risk of exposure to toxic environmental substances has drawn much attention to the possibility that Cd may be intimately involved in various pathological processes in humans. Cd exposure has a highly toxic effect on humans and animals and has been classified as a probable and potent carcinogen[20]. In the present study, adult rats were chronically exposed to different doses (1/8 or 1/4 LD_{50}) of

Fig. 28: Photomicrograph of a subgroup B (1/4 LD_{50} $CdCl_2$) liver section on injection day 45 showing maximum cytoplasmic and nuclear protein depletion. Protein content in most hepatic cells is limited to the narrow cytoplasmic areas (arrows) between unstained vacuoles (V). Bromophenol blue staining, 1000X objective

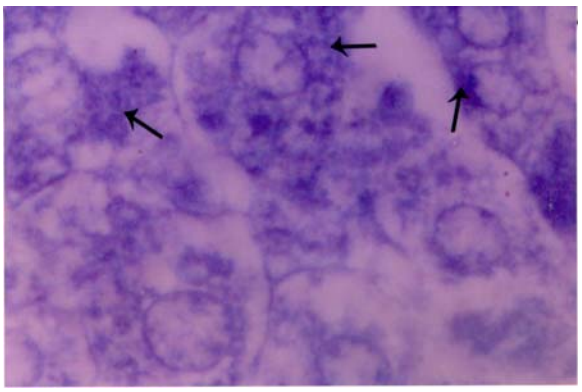

Fig. 29: Photomicrograph of a subgroup B (1/4 LD_{50} $CdCl_2$) liver section on injection day 45 and 30 days after $CdCl_2$ withdrawal showing slight restoration of nuclear and cytoplasmic total protein inclusions (arrows). Bromophenol blue staining, 1000X objective

$CdCl_2$ over 45 days in order to examine its effects on the liver. Overall, current results demonstrate marked biochemical, morphological, histopathological and histochemical changes that increased in intensity with a higher dose and/or longer exposure period. Furthermore, liver specimens examined after 30 days of $CdCl_2$ withdrawal revealed only partial recovery, which was more apparent with lower $CdCl_2$ doses and/or shorter exposure periods.

Biochemical changes: The present study demonstrated that $CdCl_2$ injection causes multiple detrimental histopathological and histochemical changes in the blood and liver. Specifically, it is found a highly significant increase in serum alanine aminotransferase, aspartate aminotransferase and alkaline phosphatase levels in both $CdCl_2$ treatment groups compared with controls. These findings agree with results observed by Masud and Nagi[9], who reported a definite rise in hepatic enzyme levels coincident with changes in normal liver histology and histochemistry.

Tissue observations: Following subcutaneous injection of $CdCl_2$ in the present study, rat livers were slightly enlarged, pale brown in color, soft in consistency. The intensity of these observations gradually increased with increasing Cd dose and/or injection period.

Histopathological changes: In the present investigation, hydropic degeneration, cytoplasmic granularity, dilatation and congestion of the central veins, as well as obliteration of the blood sinusoids, were early (injection day 20) hepatic manifestations observed following $CdCl_2$ administration. As the injection time increased, nuclear pyknosis, karyorrhexis, karyolysis and hepatic necrosis with inflammatory infiltration of the portal tracts became apparent. A longer injection period and higher $CdCl_2$ dose also revealed further accumulation of fibrous elements adjacent to the degenerated and necrotic areas which were proportional to the degree of dilatation of the blood vessels, inflammatory reaction and degeneration observed. These results agree with those of Hofflman *et al.*[21] showing acute injection of rats with Cd causes single cell necrosis, central vein congestion and swelling of some centrilobular hepatocytes. These results are also in accordance with those of Dudely *et al.*[22] in rats acutely exposed to Cd. They stated that the degree of injury progressed from generalized swelling of hepatic cells to massive necrosis with time, as well as an increased number of nucleoli and interstitial fibrosis surrounding the central vein and portal triads, after 6 months of high-dose injections. This is also consistent with reports by Katsuta *et al.*[23] and Hiratsuka *et al.*[24] in ovariectomized rats treated with Cd. In the former, increasing hepatic focal necrosis was seen after giving 3 mg of $CdCl_2$ for 3 days, while slight infiltration of lymphocytes with fibrosis of the liver capsule was noted after 25 weeks of low-dose Cd exposure in the latter.

Histochemical changes

Carbohydrates: Present results revealed marked glycogen depletion in most hepatocytes following $CdCl_2$ injection for 45 days, especially in subgroup B rats (1/4 LD_{50}). This depletion was likely due to necrotic changes produced by $CdCl_2$ injection as glycogen depletion has been previously shown to

reflect the loss of cellular capacity to metabolize glycogen normally[25]. Casarette[26] stated that necrotic changes of the liver are characterized by a lack of hepatic glycogen.

Glycogen depletion observed herein agrees with a report by Hoffman *et al.*[21], who noticed that glycogen was absent from the cytoplasm of hepatic cells obtained from mice following acute intravenous injection of Cd-acetate. Such glycogen depletion was due to accelerated glycolysis which depleted the liver of its glycogen stores. They added that chronic administration of $CdCl_2$ resulted in the relative absence of cytoplasmic glycogen granules from hepatic cells of rabbits. The mild restoration of glycogen particles in the hepatocytes of rats found in the present study 30 days after Cd withdrawal, especially those given lower $CdCl_2$ doses, however, has not yet been reported elsewhere.

DNA: The current study revealed severe depletion of chromatin bodies (DNA particles) following $CdCl_2$ injection for 45 days. These results agree with those of Nocentaini[27], who reported that Cd induces DNA damage in cultured liver cells due to free radical generation and that DNA synthesis is inhibited by chronic Cd administration. Lohmann and Beyersmann[28] reported that Cd stimulates bovine liver nuclei endonucleases, leading to DNA fragmentation.

The peripheral shift of remnant DNA particles in the nuclei of hepatocytes illustrated in the present work is in agreement with observations by El-Banhaway and Riad[29] and Sanad[30] in mammalian liver cells treated with X-ray irradiation. They regarded this phenomenon as being a distinctive sign of primary phases of karyorrhexis and karyolysis. While recovery of DNA inclusions has not yet been reported elsewhere, the present results clearly demonstrates atleast a slight recovery of DNA content in the liver in rats given high doses of Cd (1/4 LD_{50}).

RNA: Results of the current study illustrate marked loss of RNA inclusions following $CdCl_2$ injection for 45 days. These results are in accordance with those of Puvion and Lange[31], who concluded that Cd inhibits nuclear RNA synthesis. Although the loss of hepatic RNA inclusions recorded herein was partially restored 30 days after $CdCl_2$ withdrawal, further research must be conducted to confirm these findings.

Total proteins: In the current investigation, it was observed, marked protein depletion in the cytoplasm and nuclei of hepatic cells following $CdCl_2$ injection for 45 days. These results confirm those of Ovelgoenne *et al.*[32] *in vitro* on well-differentiated hepatoma. They reported that sublethal concentrations of Cd inhibit protein synthesis, which seems to be the primary factor responsible for death of hepatocytes soon after exposure to this heavy metal.

Protein depletion observed in the present study may be secondary to loss of RNA inclusions in the liver after Cd treatment as a close relationship exists between the level of RNA and protein in most animal cells under normal and pathological conditions[33]. De Vellis and Sehiede[34] reported that not only a reduction in RNA amount, but also the lesion of its functional capacity brings about such failure in protein synthesis. The depletion of total protein content in Cd-affected cells may also be due to the hyperactivity of hydrolytic enzymes as noted by Sivaprasada *et al.*[35,36].

CONCLUSION AND FUTURE RECOMMENDATIONS

It is concluded that the present study revealed that chronic $CdCl_2$ administration in adult male rats causes hepatotoxicity that intensifies with dose and duration of exposure. Rat livers displayed many lesions, hydropic degeneration, nuclear pyknosis and single cell necrosis, in addition to invasion of the portal areas and disruption of cell membranes. Moreover, the significant deterioration of parenchymal cells in the liver markedly affected their architecture and carbohydrate, DNA, RNA and total protein content and synthesis. Furthermore, 30 days after $CdCl_2$ withdrawal brought about incomplete but partial restoration of the normal hepatic structure and histochemical parameters.

Based on these results, it is recommend that industrial companies which use/deal with Cd take considerable precautions to protect workers and prevent its pollution of the surrounding environment. Since tobacco and most cigarettes contain Cd, nonsmoking programs should be a priority in reducing mortality from cardiovascular strokes and respiratory diseases. It is also recommend that all citizens and workers exposed to Cd undergo an annual checkup to monitor their liver function. Lastly, further studies are needed to better evaluate and potentially overcome the dangers associated with different forms of hazardous materials.

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