ORIGINAL ARTICLE

Dose-Dependent Morphological Changes of Cadmium Chloride on Kidney of Albino Mice

Ghumman N.A.,[1] Naseem N.,[2] Latif W.[3] and A.H. Nagi[4]
[1]*Department of Pathology, Rashid Latif Medical College*
[2,3,4]*Department of Morbid Anatomy and Histopathology, University of Health Sciences Lahore*

ABSTRACT

Background and Objectives: Exposure to certain chemicals and drugs usually exhibit marked toxic effects in living organisms. Cadmium (Cd), a heavy metal, causes nephrotoxicity and hepatotoxic agent. The objective of this study is to observe the morphological changes in the kidney of mice after exposure to cadmium chloride ($CdCl_2$).

Methodology: Albino mice (n = 48) were randomly divided into one control group and 3 experimental groups A, B and C having 12 mice in each group.In this foregoing experiment, cadmium was given as cadmium chloride orally in a dose of 5 mg/kg body weight on alternate days for 8weeks.

Results: Histopathological changes in kidneys were mainly glomerular swelling (mesangialhypercellularity), glomerular adhesions, crescentic nephropathy, tubular necrosis, thickening of the glomerular capillary wall, degeneration of renal tubules, interstitial inflammation and deposition of protein casts in renal tubules. However, the lesions were dose dependent.

Conclusion: Cadmium is a potential nephrotoxic chemical and can lead to progressive renal failure. As this chemical is a naturally occurring toxicant present in air, water, soils, and foodstuffs, hence its emission should be reduced and monitored for better healthcare of community and environment.

Keywords: Cadmium, nephrotoxicity, crescentic nephropathy, interstitial inflammation.

INTRTODUCTION

There are various causes of toxicity among which heavy metals such as Cadmium (Cd) is the most important nephrotoxic substances. Toxicants manifest their toxicity in specific target organs as reported by European Commission and the World Health Organization[1] with kidney being the most susceptible one. Cadmium was discovered by Friedrich Stromeyer in 1817 in Germany as a contaminant in Zinc carbonate.[2] It is one of the poisonous heavy metals obtained as a byproduct of mining and refining zinc.[3] Cadmium is a momentous toxin present in our environment.[4] Fundamental sources of cadmium are soil,[5] contaminated water and air by the procedure of mining, smelting and industrial waste.[6] Cadmium is enormously being utilised at conventional industrial level. The kinds of employees probably exposed to cadmium include manufacturersof alloys, aluminum, automobile, batteries, cadmium plates, ceramic and pottery, Cd-copper alloys, dental amalgam, electroplates, electrical condensers, electric instruments, glass, jewelry, luminous lamps, lithophane, pesticides, paints, photoelectric cells, pigments, plastics and many others.[7] Cadmium is taken-up from the lungs and gastrointestinal tract and is transported via blood to liver and kidneys. Within the liver, production of metallothionein is initiated by the cadmium. Cadmium that is bound to metallothionein once released into plasma will be filtered into renal tubular fluid. Metallothionein is the protein that stores cadmium in body and plays a central role in the transportation of cadmium from liver via blood to kidneys.[8] This metal accumulates within the kidneys and liver and incorporates a long biological half life of seventeen to thirty years in human being.[9] After long-term cadmium exposure, renal tubular dysfunction may develop both in experimental animals and human.[10] Cadmium exerts its toxic effects on the kidneys, respiratory system and the skeletal system and is classified as a human carcinogenby the International Agency for Research on Cancer (IARC), which is part of the World Health Organization.[4] In occupationally exposed population, preliminary signs of glomerular devastation from cadmium are escalated elimination of high mass proteins like iron binding glycoproteins and albumin. Degree of detrimental effects on glomeruli is dose-dependent and once started, the glomerular damage is believed to be irreversible.[11] In Japan, post-menopausal women who were exposed to immoderate concentrations of cadmium over their lifespan, “Itai-itai” or ouch-ouch disease was observed. In these females, the primary

Corresponding Author: Dr. Nasim Aslam Ghumman
Assistant professor; Department of Pathology, Rashid Latif Medical College, Lahore
249-B, Block-H, Marghzar Coloney, Multan Road, Lahore. Ph: 03354173718

source of cadmium exposure was through their diet, because those residential areas of Japan were significantly polluted with cadmium[12]. Signs and symptoms of "itai-itai" disease comprise of osteomalacia and alarming osteoporosis with concurrent grievous renal dysfunction.Cd levels in urine, blood, faeces, hair, kidney, liver and different tissues have been utilised as biological indicators of cadmium exposure.[13] Based on an extensive exposure to this toxic metal this study was designed to observe the morphological changes in kidneys of Albino Mice induced by different doses of oral preparations of anhydrous cadmium chloride.

MATERIALS AND METHODS

It was an experimental interventional, randomized controlled study in adult mice. Forty Eight male and female albino mice of BALB/c strain, 6 – 8 weeks old weighing 30 ± 5g, were included in the study. Animals were separated gender wise in different cages and maintained in the Animal House of the University of Health Sciences, Lahore under controlled environment (temperature 22-25˚C, humidity 65% ± 5) and light and dark cycle of 12 hours each. Albino mice were segregated in 4 groups with one control group and 3 experimental groups each comprising of 12 mice. In this foregoing experiment, cadmium was used as cadmium chloride ($CdCl_2$) orally on alternate days for 8 weeks. According to the body weight (5 mg/kg body weight) the dose was calculated and mixed with distilled water[14] (Table 1). The control group was given normal diet and plain tap water. Serum creatinine was measured at the end of the experiment by using commercially available kits (RandoxCR510, LOT: 216982). Urinary proteins were determined by strip method (Roche Diagnostic GmbH). Blood samples from each group were collected by cardiac puncture. At the end of the experiment, mice were sacrificed and kidneys were removed immediately, fixed in 10% formalin, routinely processed and stained with haematoxylin and eosin, Periodic Acid Schif staining and Periodic Acid Schiff-Methenamine Silver stain.

Statistical Analysis

The data was entered and analysed using SPSS 21.0. Mean values were taken for quantitative variables (Serum creatinine and urinary proteins). Frequencies and percentages were given for qualitative variables (histopathological changes in kidney). Fisher's exact test was applied.

RESULTS:

A total of 48 male and female albino mice of 6-8 weeks were taken. The animals were distributed into four groups with 12 mice in each group as A, B, C and control group. After a week of acclimatisation, the experiment was started. After a period of 8 weeks, the experiment was terminated and animals were sacrificed after taking blood sample via cardiac puncture. Results were analysed using Fischer's Exact test and P value was found to be significant in case of all the variables (Table 2, 3, 4).The changes noticed were mainly shown in the tables below. These include biochemical changes like proteinuria and S/Creatinine (Fig. 1-2). Histopathological changes like glomerular adhesions, glomerular crescent formation, tubular necrosis, tubular vacuolar degeneration, mesangial hypercellularity, interstitial fibrosis, vascular sclerosis, capillary wall thickness, tubular cast and interstitial inflammation. Many other histopathological changes were also noticed in the animals of group C such as glomerular sclerosis, mesangial widening, vascular congestion, perivascular inflammation, swelling of the nuclei of the tubular epithelaial cells, fibrinoid change in the afferent arterioles, lobulation of glomeruli and atrophy of glomeruli, however these changes were not observed in a significant number of animals that could be elaborated (Fig. 3-8).

Table 1: *Groups of experimental animals.*

Group	*Mice*	*Intervention*	*Dosage/ Alternate Day*	*Route*	*Duration*
Control	12	Normal diet	None	Oral	8 weeks
A	12	$CdCl_2$	5 mg/kg	Oral	8 weeks
B	12	$CdCl_2$	10 mg/kg	Oral	8 weeks
C	12	$CdCl_2$	15 mg/kg	Oral	8 weeks

Table 2:

Histological Variables				
Glomerular Adhesions				
Groups	*Absent n(%)*	*<25% n(%)*	*25-50% n(%)*	*>50% n(%)*
Control	12 (100)	0 (0.0)	0 (0.0)	0 (0.0)
A	2 (16.7)	6 (50.0)	4 (33.3)	0 (0.0)
B	0 (0.0)	6 (50.0)	6 (50.0)	0 (0.0)
C	0 (0.0)	5 (41.7)	5 (41.7)	2 (16.7)
Fisher's Exact Test = 39.689; p-value < 0.001				
Glomerular Crescent Formation				
Control	12 (100)	0 (0.0)	0 (0.0)	0 (0.0)
A	3 (25.0)	7 (58.3)	2 (16.7)	0 (0.0)
B	3 (25.0)	3 (25.0)	6 (50.0)	0 (0.0)
C	7 (58.3)	2 (16.7)	3 (25.0)	0 (0.0)
Fisher's Exact Test = 22.176; p-value < 0.001				
Tubular Necrosis				
Control	12 (100)	0 (0.0)	0 (0.0)	0 (0.0)
A	0 (0.0)	8 (66.7)	3 (25.0)	1 (8.3)
B	0 (0.0)	4 (33.3)	7 (58.3)	1 (8.3)
C	0 (0.0)	0 (0.0)	8 (66.7)	4 (33.3)
Fisher's Exact Test= 54.043;p-value < 0.001				
Tubular Vacuolar Degeneration				
Control	12 (100)	0 (0.0)	0 (0.0)	0 (0.0)
A	0 (0.0)	10 (83.3)	2 (16.7)	0 (0.0)
B	0 (0.0)	6 (50.0)	5 (41.7)	1 (8.3)
C	0 (0.0)	7 (58.3)	5 (41.7)	0 (0.0)
Fisher's Exact Test= 46.609;p-value < 0.000				

Table 3:

Mesangial Cellularity				
Groups	*Normal n(%)*	*Mild n(%)*	*Moderate n(%)*	*Severe n(%)*
Control	12 (100)	0 (0.0)	0 (0.0)	0 (0.0)
A	0 (0.0)	6 (50.0)	6 (50.0)	0 (0.0)
B	0 (0.0)	0 (0.0)	7 (58.3)	5 (41.7)
C	0 (0.0)	0 (0.0)	4 (33.3)	8 (66.7)
Fisher's Exact Test= 59.369; p-value < 0.001				
Vessel Thickness (Sclerosis)				
Control	12 (100)	0 (0.0)	0 (0.0)	0 (0.0)
A	12 (100)	0 (0.0)	0 (0.0)	0 (0.0)
B	12 (100)	0 (0.0)	0 (0.0)	0 (0.0)
C	6 (50.0)	6 (50.0)	0 (0.0)	0 (0.0)
Fisher's Exact Test= 32.619;p-value < 0.005				

Table 4: *Glomerular Capillary Wall Thickness after 8 Weeks.*

Groups	*Normal n(%)*	*Focally Thick n(%)*	*Diffusely Thick n(%)*
Control	12 (100)	0 (0.0)	0 (0.0)
A	4 (33.3)	8 (66.7)	0 (0.0)
B	2 (16.7)	3 (25.0)	7 (58.3)
C	0 (0.0)	3 (25.0)	9 (75.0)
Fisher's Exact Test= 42.45; p-value < 0.019			

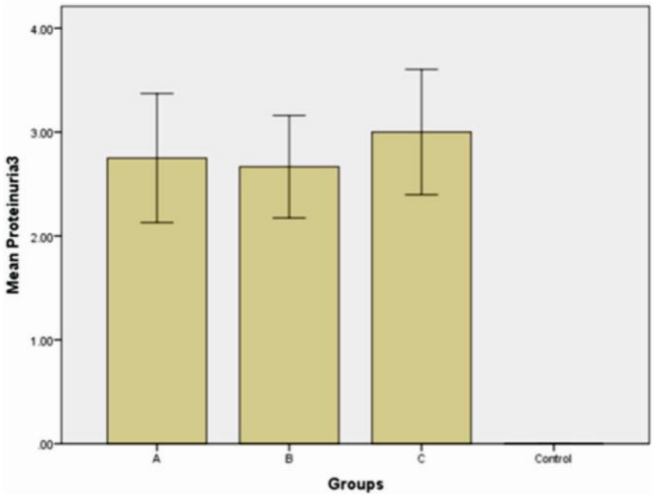

Fig. 1: *Proteinuria (mg/dl) after 8 Weeks of the Experiment*

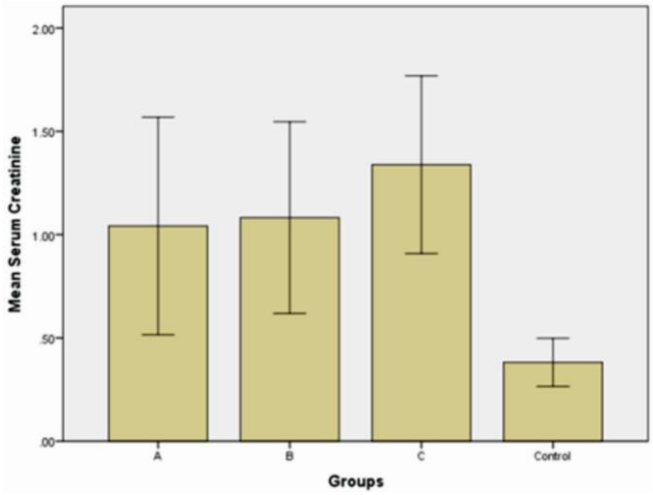

Fig. 2: *Serum Creatinine after 8 Weeks of the Experiment.*

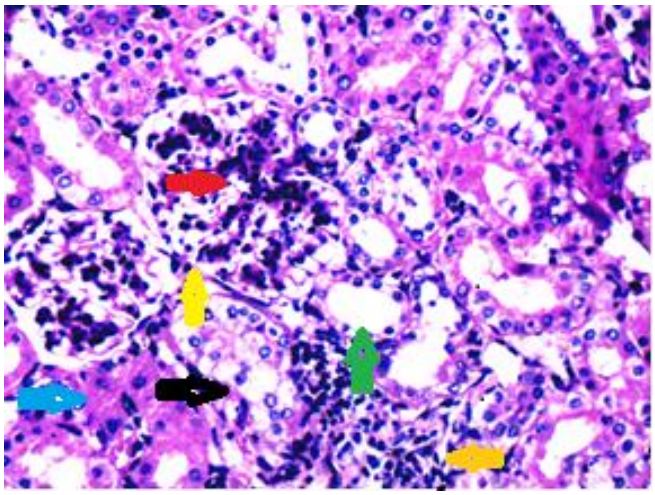

Fig. 3: *Photomicrograph showing glomerular adhesions (yellow arrow), mesangialhypercellularity (red arrow), tubular dilatation (green arrow), tubular vacuolar degeneration (black arrow), interstitial inflammation (orange arrow) and interstitial fibrosis (blue arrow). (20 X H&E).*

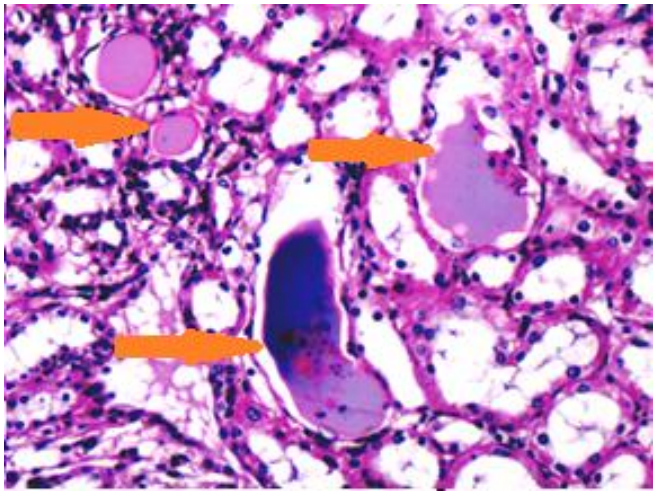

Fig. 4: *Photomicrograph showing cast (red arrow) in medullary tubules. (20 X H&E).*

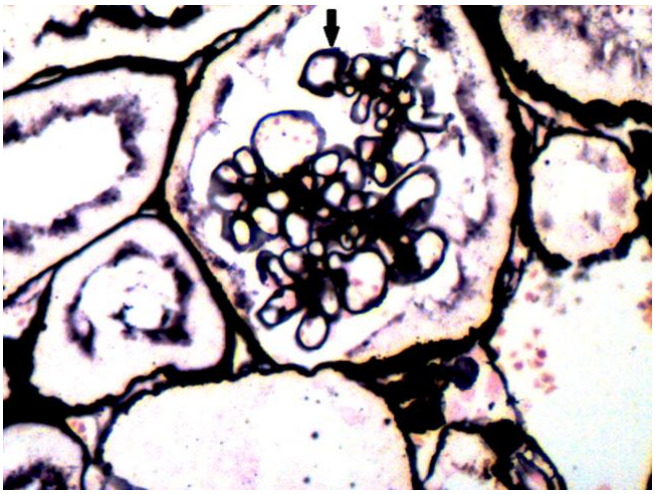

Fig. 5: *Photomicrograph showing glomerular capillary wall thickness (black arrow). (40 X JMS).*

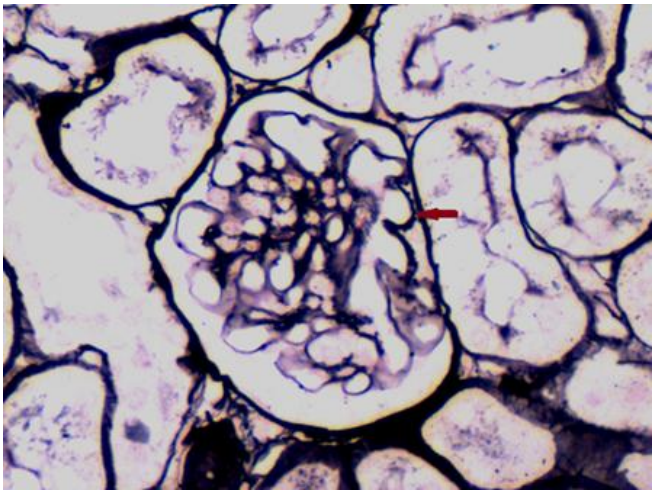

Fig. 6: *Photomicrograph showing deposits in glomerular basement membrane (red arrow). (40 X JMS).*

DISSCUSSION

Cadmium is a toxic metal that is present throughout the environment. In humans and animals, it accumulates primarily in kidneys and liver.[15] In mammals, diet is the major route of exposure through which they are exposed to toxic metals. Important organs significantly kidney and liver are the fundamental target sites.[16] To evaluate the metal toxicity, histopathological lesions are thought of as biomarkers. Histological biomarkers are of paramount significance as the particular target organs which are responsible for important functions are examined properly. Furthermore, the alterations perceived in these fundamental organs are generally very simple to appreciate as compared to functional ones and can also be used as alarming signs for healthiness of organisms. The current experiment was designed to determine the dose-dependent morphological effects of oral $CdCl_2$ in albino mice over 8 weeks duration. The purpose of this study was to provide an essence to understand the similar pathological lesions in humans. In this study, significant association has been found between dose of $CdCl_2$ and proteinuria. After 8 weeks, 8 mice each from group C and B showed proteinuria of 100 mg/dl whereas 2 animals from group C also developed proteinuria of 500mg/dl (Figure 1). Similar results have been observed in past studies that show that proteinuria is the earlier indication of kidney destruction.[17] Regarding serum creatinine, significant number of animals showed rise in serum creatinine level after 8 weeks. Eight mice each from group C and B developed serum creatinine within 1.00-1.50 mg/dl whereas 2 animals from group C also developed

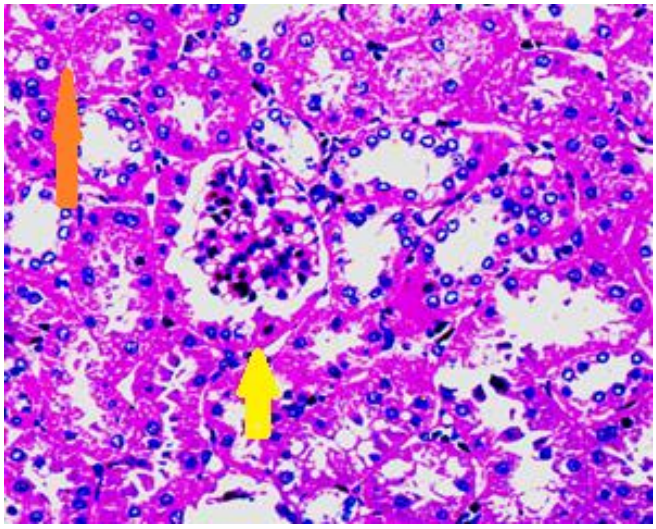

Fig. 7: *Photomicrograph showing tubular necrosis (red arrow) and crescent formation (yellow arrow). (20 X H&E).*

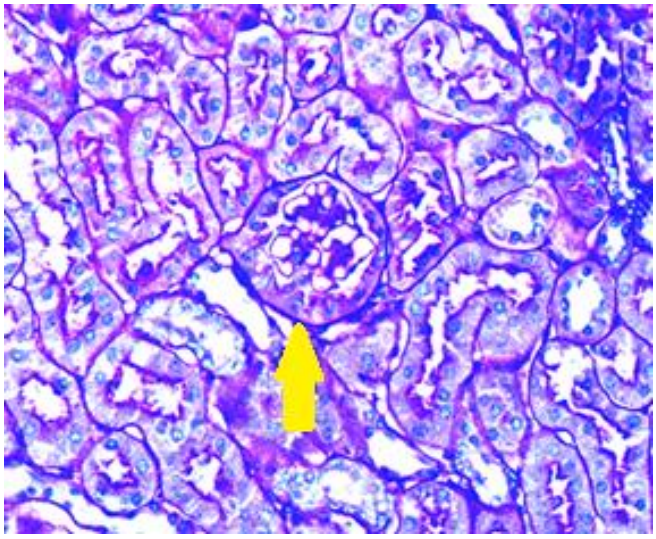

Fig. 8: *Photomicrograph shows crescent formation (yellow arrow). (20 X PAS)*

S/Creatinine within 1.51-2.50 mg/dl (Figure 2). Elevated levels of S/Creatinine after Cd exposure were observed by Abdel-Moneim and Said in 2007[18]. This study showed that higher number of mice from group C developed remarkable glomerular damage within the kidney which include glomerular adhesions and mesangial heypercellularity (Figure 3) as explored by other studies by different other researchers.[19] Dose dependent thickening of the capillary walls was found to be present more in group C, where diffuse thickness was observed greater than at focal level, followed by group B and A, with focal and diffuse thickness (Figure 5). Thickening of the basement membrane of glomerular capillaries has been described earlier once Cd exposure.[20] Damage at tubular level is also seen in this study which includes tubular necrosis and vacuolar degeneration. Here, again the damage remained dose dependent showing > 50% necrosis in mice of group C, B and A, as well as a few of them showed tubular necrosis upto 25-50% (Figure 7)and these consequence of cadmium are well established.[21] Regarding tubular vacuolar degeneration, it was also found in all the experimental mice. This damage was in accordance with the other results mentioned above with more damage in group C followed by B and A (Figure 3) as described by other researchers.[22,18,20] In mice, cadmium also induced deposition of eosinophilic casts, mainly protein cast in the tubules (Figure 4). Severe interstitial inflammation was also a commendable observation in this study. The degree of interstitial inflammation was exactly in accordance with the other results of this study revealing that it was dose dependent in all groups. So majority of the group C animals were found to develop moderate to severe inflammation (Figure 3) as narrated in the past studies by Prozialeck et al. in 2009.[19] Blood vessels were also not spared and developed thickness of their wall (sclerosis). Vascular sclerosis by the cadmium toxicity have been explored by Messner and Bernhard (2010)[23] that showed that cadmium is a distinctive risk factor for vascular disease.

It is ***concluded*** that Cadmium is a potential nephrotoxic chemical and can lead to progressive renal failure by inducing dose-dependent pathological changes in glomeruli. In this study pathological changes in proximal tubules, interstitium and blood vessels are also observed culiminating to tubulointerstitial fibrosis. As this chemical is a naturally occurring toxicant present in air, water, soils, and foodstuffs, hence its emission may be reduced and monitored for better healthcare of community and environment.

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Author's Contribution

NAG: Substantial contribution of concept and design of work, write-up, analysis and data interpretation and results compiling. NN: Supervision and revising it critically for important intellectual content. WL: Help in biostatics and data analysis. AHN: Overall supervision in concept and design of work and final approval of the work.

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University of Health Sciences, Lahore.

Conflict of Interest: There is no conflict of interest.

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