Original Article

Effect of Cadmium Chloride on Kidney

Alterations in Serum Creatinine and Urinary Proteins in Albino Mice After Exposure to Different Doses of Anhydrous Cadmium Chloride Via Oral and Intraperitoneal Routes

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ABSTRACT

Objective: Cadmium (Cd), a heavy metal has a potential to develop toxicity in various organs especially kidney and liver. Serious toxicity of cadmium became the centre of attention because of its association with neoplastic and non-neoplastic lesions in various organs and tissues. The objective of this study was to evaluate the detrimental effects of cadmium on kidney leading to increased urinary proteins and serum creatinine.

Study Design: An experimental study on albino mice

Place and Duration of Study: This study was conducted at the Department of Morbid Anatomy and Histopathology; University of Health Sciences, Lahore for 8 weeks in the year 2014.

Materials and Methods: Albino mice (n=72) were randomly divided in groups as control group and 5 experimental groups A, B, C, D and E with 12 mice in each group. In this experiment, cadmium was used as anhudrous cadmium chloride (CdCl2) orally and intraperiotoneally on alternate days for 8 weeks in a dose of 5mg/kg body weight. Bovine serum albumin was also used once in group D to induce serum sickness leading to development of glomerular damage.

Results: This experimental work revealed the biochemical alterations in kidney like proteinuria and serum creatinine because of cadmium. However, these biochemical changes were proportional to the dose and route of introduction of cadmium chloride.

Conclusion: Cadmium is a toxic heavy metal that can lead to progressive renal failure. Cadmium toxicity leads to progressive damage to glomeruli. In this study biochemical changes were observed that were proportional to different doses of cadmium. As this chemical is a naturally occurring toxicant that exists everywhere in air, soils, foodstuff and water, hence to control the emission of this toxicant should be of high priority for better healthcare of community.

Key Words: Nephrotoxicity, proteinuria, intraperitoneal, Bovine serum albumin, serum sickness.

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INTRODUCTION

Humans frequently come across a variety of noxious substances that are probably toxic for kidney. Heavy metal noxious agents such as lead, cadmium, mercury, copper, uranium, and bismuth are some of the environmental nephrotoxins to which humans are exposed[1]. Contact with heavy metals is potentially harmful.

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As the kidney has the ability to reabsorb and gather metals with a valence of two, therefore kidney is the chief target organ of heavy metal toxicity[2]. Cadmium is a momentous toxin present in our environment[3]. Cadmium was discovered by Friedrich Stromeyer[4] and Karl Samuel Leberecht Hermann in 1817 in Germany as a contaminant in zinc carbonate[5]. Cadmium is enormously being utilized at conventional industrial level, as it is an essential constituent in production of batteries, predominantly in rechargeable nickel-cadmium batteries and is present in metal pigments and coatings and is commonly used in electroplating[6]. Cd is also utilized as a barricade to modulate neutrons in nuclear fission[7]. Cd and its oxides have been utilized in black and white television phosphorous and in the green and blue phosphorous for image tubes in colour television[8].

Cadmium exists in air as fine particulate, less than 10μm in size. Cd particulate is disseminated by air and ultimately either settles down by rain or snow or as dry deposits on ground or surface water. The fine

particulate of metal may persist in air for days to weeks and are carried away for thousands of kilometers. Cd occurs either dissipated or as part of indissoluble complexes in water. Soluble form of this metal is ambulant in water and in soil. A crucial source of cadmium in soil is from the phosphate fertilizers which is used for agricultural motives. Cadmium gets accumulated in plants, in root vegetables and shoots like rice, wheat, tobacco, peanuts or cocoa and also in animals like offal, mollusks and crustaceans[9]. Other sources are food, alcoholic beverages and cigarette smoking[10]. Cadmium is predominantly found in fruits and vegetables due to its high rate of soil-to-plant transfer[11]. Biological half-life of Cd is long in individuals and gets assembled in fundamental organs, principally in kidney and liver[12]. Cd levels are elevated in mushrooms and shellfish[13].

On the background of the statistics procured from human occupational exposure, Cd and Cd containing complexes were classified as group one human carcinogens by the International Agency for Research on Cancer (IARC) in 1993, which is part of the World Health Organization[3].

The general population comes across Cd by various routes. Injection is one of the major routes. There are certain areas where soil is momentously contaminated with Cd in the Jinzu and Kakehashi river valleys in Japan. In these regions, rice absorb metal from the soil and eventually lifetime eating of these rice contaminated with Cd, can cause grievous kidney and bone disease that is known as "Itai-Itai" illness, predominantly in females[14,15]. Inhalation is the predominant route of exposure in occupationally exposed population[13].

Data from experiments on animals demonstrates that initially after exposure, cadmium in blood is bound to albumin and is particularly taken up by the liver. Within the liver, production of metallothionein is instigated by the cadmium. Metallothionein is a protein with low molecular weight and is involved in cadmium, zinc and copper metabolism. It acts as a detoxifying agent for cadmium and plays a central role in the transportation of cadmium from liver via blood to kidneys. It has a molecular weight of around 6500Da. Metallothionein may serve in a protective way by binding cadmium in a stable bio complex. In this way interference of cadmium with other cellular components is decreased and the acute effects normally seen after larger and acute exposure can be prevented[16].

Cadmium exposure can lead to both acute and chronic intoxications[17]. After ingestion of higher concentrations of cadmium, the symptoms in the gastrointestinal tract include nausea, vomiting, abdominal pain, cramps, tenesmus and diarrhea[18]. If cadmium-contaminated air is inhaled, it can lead to damage to mucous membranes. Other serious effects like shortness of breath, pulmonary oedema, pulmonary inflammation and emphysema can occur[19]. Among smokers, development of chronic obstructive disease due to cadmium content in smoke has also been observed in various studies[20]. Chronic inhalation of cadmium is also presumed to be a probable cause of lung carcinomas[21,22] and evolution and progression of peripheral vascular disease[23].

Nephrotoxicity by cadmium may develop as a consequence of chronic ingestion or inhalation. In occupationally exposed population, prefatory signs of glomerular damage 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[13]. Substantial cadmium exposure may also be a cause of diminished glomerular filtration rate and chronic renal failure. Cadmium induced nephrotoxicity has been reported in environmental pollution and industrial exposure[24].

Cadmium accumulates mainly in the proximal tubules of the kidney and causes kidney dysfunction after chronic exposure[25]. The noxious effects of cadmium on the cells of proximal tubules cause decreased reabsorption of low molecular weight proteins that ultimately results in increased excretion of these proteins in urine, so-called 'tubular proteinuria[26].

MATERIALS AND METHODS

It was an experimental interventional, randomized controlled study in adult mice. Seventy-two 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 6 groups with one control group and 5 experimental groups each comprising of 12 mice. In this foregoing experiment, cadmium was used as cadmium chloride (CdCl2) orally and itraperitoneally on alternate days for 8 weeks. According to the body weight (5mg/kg body weight) the dose was calculated and mixed with distilled water (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 (Randox CR510, LOT: 216982). Urinary proteins were determined by strip method (Roche Diagnostic GmbH). Blood samples from each group were collected by cardiac puncture. At the commencement of the experiment, proteinuria was measured of all the animals of all six groups. All the animals showing proteinuria even in traces were rejected and those animals were selected who showed no proteinuria. During the experiment proteinuria was taken as: at the end of 3 weeks, 5 weeks and 8 weeks.

Statistical Analysis: The data was entered and analyzed using SPSS 21.0. Mean ± S.E.M was given for quantitative variables (Serum creatinine and urinary proteins). Fisher exact test was applied.

RESULTS

72 male and female albino mice of 6-8 weeks age were selected and distributed into six groups with 12 mice in each group as A, B, C, D, E and control group. The experiment was started after one week of acclimatization. Urinary proteins were checked after 3, 5 and 8 weeks of experiment. Experiment was terminated after 8 weeks. Animals were sacrificed after taking blood sample for serum creatinine via cardiac puncture. Results were analyzed using Fischer's Exact test and P value was found to be significant (Table 2,3). Alterations in urinary proteins and S/Creatinine are shown in the tables below.

Table No.1: Groups of experimental animals

Group	Mice	Intervention	Dosage/Alternate day	Route	Duration
Control	12	Normal diet	None	Oral	8 weeks
A	12	$CdCl_2$	5mg/kg	Oral	8 weeks
B	12	$CdCl_2$	10mg/kg	Oral	8 weeks
C	12	$CdCl_2$	15mg/kg	Oral	8 weeks
D	12	BSA(single dose)+ $CdCl_2$	250mg/kg + 10mg/kg	Intraperitoneal + Oral	8 weeks
E	12	$CdCl_2$	10mg/kg	Intraperitoneal	8 weeks

Table No.2: Proteinuria(Mg/Dl) at the Start of the Experiment

Groups	Nil n(%)	Traces n(%)	30 n(%)	100 n(%)	500 n(%)	Total n(%)
Control	12(100)	0(0.0)	0(0.0)	0(0.0)	0(0.0)	12(100)
A	12(100)	0(0.0)	0(0.0)	0(0.0)	0(0.0)	12(100)
B	12(100)	0(0.0)	0(0.0)	0(0.0)	0(0.0)	12(100)
C	12(100)	0(0.0)	0(0.0)	0(0.0)	0(0.0)	12(100)
D	12(100)	0(0.0)	0(0.0)	0(0.0)	0(0.0)	12(100)
E	12(100)	0(0.0)	0(0.0)	0(0.0)	0(0.0)	12(100)
Total	72(100)	0(0.0)	0(0.0)	0(0.0)	0(0.0)	72(100)
Proteinuria (mg/dl) after 3 weeks of the experiment						
Control	12(100)	0(0.0)	0(0.0)	0(0.0)	0(0.0)	12(100)
A	4(33.3)	0(0.0)	8(66.7)	0(0.0)	0(0.0)	12(100)
B	2(16.7)	1(8.3)	9(75.0)	0(0.0)	0(0.0)	12(100)
C	2(16.7)	0(0.0)	10(83.3)	0(0.0)	0(0.0)	12(100)
D	0(0.0)	0(0.0)	7(58.3)	5(41.7)	0(0.0)	12(100)
E	1(8.3)	0(0.0)	6(50.0)	5(41.7)	0(0.0)	12(100)
Total	21(29.2)	1(1.4)	40(55.6)	10(13.9)	0(0.0)	72(100)
Statistical Analysis: Fisher's Exact Test: 52.870; P Value: 0.000 (<0.001)						
Proteinuria (mg/dl) after 5 weeks of the experiment						
Control	12(100)	0(0.0)	0(0.0)	0(0.0)	0(0.0)	12(100)
A	0(0.0)	4(33.3)	7(58.3)	1(8.3)	0(0.0)	12(100)
B	0(0.0)	2(16.7)	3(25.0)	7(58.3)	0(0.0)	12(100)
C	0(0.0)	2(25.0)	1(8.3)	9(75.0)	0(0.0)	12(100)
D	0(0.0)	0(0.0)	0(0.0)	12(100)	0(0.0)	12(100)
E	0(0.0)	0(0.0)	1(8.3)	11(91.7)	0(0.0)	12(100)
Total	12(16.7)	8(11.1)	12(16.7)	40(55.6)	0(0.0)	72(100)
Statistical Analysis: Fisher's Exact Test =76.057; P =0.000 (<0.001)						
Proteinuria (mg/dl) after 8 weeks of the experiment						
Control	12(100)	0(0.0)	0(0.0)	0(0.0)	0(0.0)	12(100)
A	0(0.0)	0(0.0)	4(33.3)	7(58.3)	1(8.3)	12(100)
B	0(0.0)	0(0.0)	4(33.3)	8(66.7)	0(0.0)	12(100)
C	0(0.0)	0(0.0)	2(16.7)	8(66.7)	2(16.7)	12(100)
D	0(0.0)	0(0.0)	0(0.0)	2(16.7)	10(83.3)	12(100)
E	0(0.0)	0(0.0)	0(0.0)	2(16.7)	10(83.3)	12(100)
Total	12(16.7)	0(0.0)	10(13.9)	27(37.5)	23(31.9)	72(100)
Statistical Analysis: Fisher's Exact Test : 83.689; P Value: 0.000 (<0.001)						

Table No.3: Serum Creatinine After 8 Weeks of the Experiment

Groups	0.30-0.59 mg/dl n(%)	1.00-1.50 mg/dl n(%)	1.51-2.50 mg/dl n(%)	Total n(%)
Control	12(100)	0(0.0)	0(0.0)	12(100)
A	4(33.3)	7(58.3)	1(8.3)	12(100)
B	4(33.3)	8(66.7)	0(0.0)	12(100)
C	2(16.7)	8(66.7)	2(16.7)	12(100)
D	0(0.0)	2(16.7)	10(83.3)	12(100)
E	0(0.0)	2(16.7)	10(83.3)	12(100)
Total	22(30.6)	27(37.5)	33(31.9)	72(100)
Statistical Analysis: Fisher's Exact Test = 63.851; P = 0.000 (<0.001)				

Table No.4: Mean + S.E.M of Serum Creatinine

Groups	N	Mean	Std. Error
Control	12	.3817	.03362
A	12	1.0417	.15204
B	12	1.0825	.13393
C	12	1.3383	.12410
D	12	1.9358	.08553
E	12	2.0700	.10054
Total	72	1.3083	.08079

Serum creatinine was also analysed using ANOVA test. P value (<0.005) was found to be significant between and within the groups showing the significant difference in values of serum creatinine (Table 3). Standard error of mean (S.E.M) is shown in the bar chart 1.

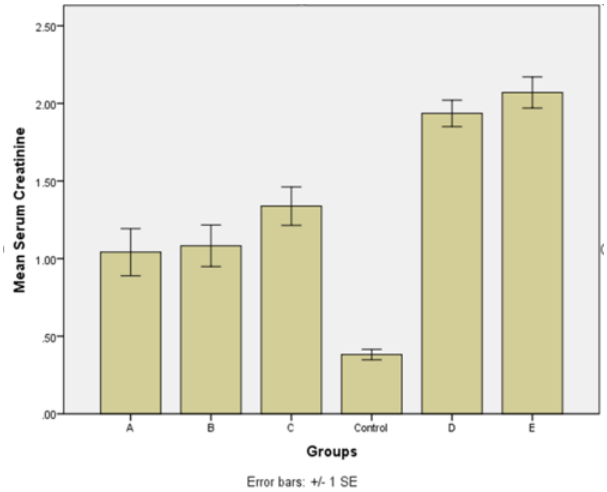

Figure No.1: Man Serum Creatinine

DISCUSSION

Cadmium is a toxic metal that is present throughout the environment. In humans and animals, it accumulates primarily in kidneys and liver. 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. The current experiment was designed to appraise the biochemical effects of $CdCl_2$ via oral and intraperitoneal route in totally different concentrations for 8 weeks. The purpose of this study was to provide an essence to understand the similar biochemical changes in humans. In this study, significant association has been found between dose of $CdCl_2$ and proteinuria.

Significant number of mice developed proteinuria after oral exposure that was dose dependent. After 3 weeks, 40 mice showed proteinuria of 30mg/dl and among them the majority of the mice were from group C, B and A (oral groups). After 5 weeks, 9 mice from group C, 7 mice from group B, and 1 from group A, developed proteinuria of 100mg/dl, that shows clear association between groups and oral dose of CdCl2. This association was maintained after 8 weeks where proteinuria of 500mg/dl was observed in 2 animals of group C and 1 animal of group A. Proteinuria of 100mg/dl was seen in 8 mice each from group C and B and 7 mice of group A. Regarding the role of BSA and intraperitoneal routes, after 3 weeks proteinuria of 100mg/dl was observed in 5 mice from group D and 5 mice from group E. After 5 weeks duration, proteinuria was found in a significant number of mice that were 40. The majority of the animals was from group D and E, including all 12 mice of group D and 11 mice of group E, followed by group C, B and A with 9, 7 and mice respectively. After 8 weeks duration of the dose of CdCl2, proteinuria of 500mg/dl was observed in a remarkable number of mice that were 23 in a total where 27 mice showed proteinuria of 100mg/dl. Again the majority was from group D and E with 500mg/dl proteinuria having 10 mice from each group. Hence this study shows that the mice which were given intraperitoneal dose of 250mg BSA once at the start of the experiment, followed by 10mg CdCl2/kg body weight on alternate days, developed renal damage earlier and more in severity than the other groups which were receiving oral dose only. Therefore, this experiment shows that BSA produces autoimmunity in the form of serum sickness with increased capillary permeability causing increased vulnerability of glomeruli to damage by toxicants. It was also found that group E, receiving CdCl2 via intraperitoneal route on alternate days, developed equivocal renal damage more severe than the oral groups. Therefore, these findings

are consistent with the other studies describing that the earlier indication of kidney devastation is generally proteinuria[27].

This study also showed that there was a significant rise in serum creatinine levels among the animals. Normal range of serum creatinine is 0.43mg/dl + 0.14mg/dl in male mice and 0.45mg/dl + 0.07mg/dl in female mice[28]. Remarkable rise in serum creatinine levels were observed in animals of group D and E. Ten animals from group D and ten from group E showed serum creatinine level up to 2.50gm/dl. Although these findings are parallel to damage to glomeruli that lead to proteinuria. Elevated levels of serum creatinine after cadmium exposure were observed by Abdel-Moneim and Said[29].

CONCLUSION

This study suggests that cadmium is one of the noxious heavy metals that can leads to toxicity in kidneys resulting in proteinuria and raised serum creatinine levels. These biochemical changes were observed to be proportional to different doses and routes of cadmium. Since cadmium is a cumulative toxin, the most important recommendation is to minimize or avoid known sources of exposure to cadmium.

Author's Contribution:

Concept & Design of Study:	Nasim Aslam Ghumman
Drafting:	Nosheen Khurrum, Farwa Shamsi
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Revisiting Critically:	Nasim Aslam Ghumman, Nosheen Khurrum
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Conflict of Interest: The study has no conflict of interest to declare by any author.

REFERENCES

1. Yu CC, Lin JL. and Lin-Tan DT. Environmental exposure to lead and progression of chronic renal diseases: A four-year prospective longitudinal study. J Am Soc Nephrol 2004;15(4):1016–1022.
2. Ferguson CJ, Wareing M, Ward D, Green R, Smith CP, Riccardi D. Cellular localization of divalent metal transporter DMsT-1 in rat kidney. Am J Physiol Renal Physiol 2001;280:F803–F814.
3. Satarug S, Garrett SH, Sens MA. and Sens DA. Cadmium, environmental exposure, and health outcomes. Environ. Health. Perspect 2010;118: 182–190.
4. Hermann CS. Noch ein schreiben über das neue Metall. Annalen der Physik 1818;59(5):113.
5. Morrow H. Cadmium and Cadmium Alloys". Kirk-Othmer Encyclopedia of Chemical Technol 2010; 1–36.
6. Martin S, Griswold W. Human health effects of heavy metals. Environmental Science and Technology Briefs for Citizens 2009;(15):1–6.
7. Scoullos MJ. Mercury, Cadmium, Lead: Handbook for Sustainable Heavy Metals Policy and Regulation. Springer 2001;104–116.
8. Ching-Hwa L, Hsi CS. Recycling of Scrap Cathode Ray Tubes. Environ Sci Tech 2002;36(1):69–75.
9. Jarup L, Akesson A. Current status of cadmium as an environmental health problem. Toxicol Appl Pharmacol 2009;238:201–208.
10. Menke A, Muntner P, Silbergeld EK, Platz EA, Guallar E. Cadmium levels in urine and mortality among U.S. adults. Environ Health Perspect 2009;117:190–196.
11. Satarug S, Garrett SH, Sens MA, Sens DA. Cadmium, environmental exposure, and health outcomes. Ciência & Saúde Coletiva 2011; 16(5):2587–2602.
12. Ye JL, Mao WP, Wu AL, Zhang NN, Zhang C, Yu YJ, Zhou L, et al. Cadmium-induced apoptosis inhumannormal liver L-02 cells by acting on mitochondria and regulating Ca2+ signals. Environ. Toxicol. Pharmacol 2007;24:45–54.
13. Jarup L. Cadmium overload and toxicity. Nephrology Dialysis Transplantation.17(Suppl 2).2002;35-39.
14. Ezaki T, Tsukhara T, Moriguchi J, Furuki K, Fukui Y, Ukai H, et al. No clear-cut evidence for cadmium-induced renal tubular dysfunction among over 10,000 women in the Japanese general population: a nationwide large-scale survey. Int Archives of Occupational and Environmental Health 2003;76: 186-196.
15. Kobayashi E, Suwazono Y, Uetani M, Kido T, Nishijo M, Nakagawa H, et al. Tolerable level of lifetime cadmium intake estimated as a benchmark dose low, based on excretion of β2-Microglobulin in the cadmium-polluted regions of the Kakehashi River Basin, Japan. Bull. Environ. Contam Toxicol 2006;76: 8-15.
16. Klassen CD, Liu J. and Diwan BA.Metallothionein protection of cadmium toxicity. Toxicol Appl Pharmacol 2009;238:215–220.
17. Chakraborty S, Dutta AR, Sural S, Gupta D, Sen S. Ailing bones and failing kidneys: a case of chronic cadmium toxicity. Ann Clin Biochem 2013;50(Pt 5):492-5.
18. Nordberg GF. Cadmium and health in the 21st century—historical remarks and trends for the future. Biometals 2004;17(5): 485-489
19. Kirschvink N, Martin N, Fievez L, Smith N, Marlin D, Gustin P. Airway inflammation in cadmium-exposed rats is associated with pulmonary oxidative stress and emphysema. Free Radic Res 2006;40(3):241–250.

20. Agency for Toxic Substances & Disease Registry (ATSDR). Toxicological Profile for Cadmium. Atlanta, GA, U.S. Department of Health and Human Services, Public Health Service 2012.
21. Verougstraete V, Lison D. Cadmium, lung, and prostate cancer: a systematic review of recent epidemiological data. J Toxicol Environmental Health 2003;6(Part B): 227-255.
22. Sorahan T, Esmen N. Lung cancer mortality in UK nickel-cadmium battery workers, 1947-2000." Occup. Environ Med 2004;61:108-116.
23. Navas-Acien A, Silbergeld E, Sharrett R, Calderon-Aranda E, Selvin E, Guallar E. Metals in Urine and Peripheral Artery Disease. Environ Health Perspect 2005;113(2):164-169.
24. Gonick HC. Nephrotoxicity of cadmium & lead. Ind J Med Res 2008;128:335–52.
25. Johri N, Jacquillet G, Unwin R. Heavy metal poisoning: the effects of cadmium on the kidney. Biometals 2010;23:783–92.
26. Bernard A. Renal dysfunction induced by cadmium: biomarkers of critical effects. Biometals 2004;17:519–23.
27. Thomas LD, Hodgson S, Nieuwenhuijsen M, Jarup L. Early kidney damage in a population exposed to cadmium and other heavy metals. Environ. Health. Perspect 2009;117: 181–184.
28. Fox JG, Anderson LC, Loew FM, Quimby FW, editors. Laboratory Animal Medicine, 2nd ed. 2002. p.44.
29. Abdel-Moneim AM, Said KM. Acute effect of cadmium treatment on the kidney of rats: biochemical and ultrastructural studies. Pak J Biol Sci 2007;10:3497–3506.