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Studies on Hepato and Renal Toxicity of Cadmium on Normal and Protein Malnourished Rats

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Abstract

Cadmium is an established toxic metal with its ability to accumulate in blood, liver and kidney. It adversely affects the renal cortex and ultimately leads to the renal failure. It has been reported that deficiency of certain dietary components affects cadmium toxicity. Present study was designed to evaluate the cadmium toxicity (50 ppm in drinking water) in protein malnourished group (8%) and on normal protein (21%) diet groups. It was found that Cd exposure led to marked elevation in alkaline phosphatase, acid phosphatase, glutamic pyruvic transferase, glutamic oxaloacetic acid transferase with significant increase in protein malnourished group (p 0.005).

Same trend of result was noted in urinary concentration of alpha amino acid, albumin, total protein and glucose. Results were more significant (p 0.001) in malnourished animals. However, hepatic and renal enzymes showed depletion in all groups. There was marked increase in serum enzymes GOT, GPT, alkaline phosphatase, acid phosphatase in low protein diet + Cd (p 0.001).

Hence present findings strongly support that protein under-nutrition predisposes the organism to the deleterious effects of cadmium toxicity.

Keywords: Cadmium; Protein malnourishment; Renal toxicity; Hepato toxicity

Introduction

Various metals, in trace amounts, are essential for proper biological functioning. However, there are many heavy metals that are toxic and exposure to their high intake may result in serious adverse effects on health. Cadmium (Cd) is one of the most toxic pollutants in environment. Accumulation of Cd in blood affects the kidney and liver. The toxicity of cadmium is much affected by deficiency of certain dietary components.

Here we propose to study the effect of protein malnutrition on cadmium toxicity in albino rats. Such a study may be of help to safeguard the health of industrial workers as well as public at large.

An enteropathy has been observed in patient with Itai-Itai disease [1] and in Cadmium fed experimental animals [2,3]. It appears that under given conditions, a critical concentration of cadmium in the mucosal cells, produces structural damage, accompanied by marked changes in absorption of cadmium and other dietary components [4].

Kidney and liver are most affected by cadmium toxicity. The critical organs in man with respect to cadmium metabolism appear to be the kidney. Friberg et al. postulated that when the concentration of cadmium reaches a critical level (200 µg /g) in the renal cortex renal tubular damage occurs [5]. Alteration in antioxidant defense system in the rat testes was found with Cd exposure [6]. Studies showed toxic nephropathy might be detectable in an early stage by assay of the enzymes in urine. Cd exposure leads to decrease in glutamate, aspartate, glutamine, GABA and taurine content of rat striatum [7]. Cadmium chloride ($CdCl_2$), administered during gestation period on female wistar rats resulted in decrease in body weight gain and induced hepatotoxicity [8]. Glomerular injury is suggested by increased activities of ACP in urine and injury to proximal tubules by elevation of ALP, the main target organs being kidney and liver [9].

Vitamin D-deficient diet on chronic cadmium exposure in rats might lead to adverse effects [10]. Previously, it was found that cadmium chloride , 0.25 Cd/kg for 5 days a week, decreased alkaline phosphatase

activity of the renal cortex at 23rd weeks [11], and there was a decrease in the capacity to reabsorb glucose, together with considerable proteinuria and excretion of cadmium [12,13]. Wilson induced anemia in rats with a cadmium diet; increase in eosinophils and reticulocytes with hyperplastic bone marrow were also found [2]. Marked decrease in hemoglobin was found in rats, treated with 50 ppm Cd, in drinking water, Cadmium created a state of iron deficiency without any blockage in hemoglobin synthesis or erythropoietic activity [14].

It has been proved by many researchers that cadmium is a potent and cumulative toxic metal. Its toxic effects on experimental animals as well as human subjects have been well studied. It is also known that the nutritional status of animal is a significant factor in determining the degree of cadmium poisoning.

Protein malnutrition is an uncontrolled public health problem, particularly in developing countries and nutritional factors are known to play a great role in individual susceptibility to the neurotoxic effects of environmental chemicals [15]. Nutritional deficiencies are known to alter the response of the organism to the environmental toxicant in a manner different to that observed in the nutritional adequate state. Same type of study is done in rhesus monkeys (*Macaca mulatta*) in relation to protein calorie malnutrition [15]. The vitamin D-deficient diet decreased serum concentration of vitamin D, but it did not affect the metabolism of the kidney or bone. Cadmium treatments alone induce a decrease in serum concentration of vitamin D, as well as renal dysfunction, renal anemia, and abnormal bone metabolism [10].

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Hence a long term study (120 days) was conducted in growing rats exposed to cadmium (50 ppm, in drinking water). The effects were assessed in normal (21% protein diet) and protein malnourished (8% protein diet).

Materials and Methods

All chemicals/ reagents used for these experiments were of high quality research grade. All major chemicals were purchased from Sigma Chemical Co. (USA) and olive oil was purchased from the local supplier. Biochemical estimations were carried by using kits, supplied by Bio Lab Diagnostic Pvt. Ltd.

Experimental animals

160 weaned male rats of a wistar–derived strain, about 25- 35gm, were randomly allocated into four groups of 40 rats in each: Group (I) 21% protein diet + drinking water (control), (II) 21% protein diet + drinking water (Cd, 50 ppm), (III) 8% protein diet + drinking water (control) and (IV) 8% protein diet + drinking water (Cd, 50 ppm).

The animals were fed with standard Wetherholtz diet, had free access to water under well ventilated condition of 12h light cycle. The animals were adapted to laboratory condition for 7 days prior to the experiments. Investigations using experimental animals were conducted in accordance to the Organization for Economic Cooperation and Development guidelines no. 407 (OECD, Paris, 1993). The studies were performed with the approval of Institutional Animal ethics committee (IAEC).

Biochemical parameters

Experiments were conducted to study mostly hepatic and renal toxicity. The following parameters were chosen for assessing the hepatotoxicity: Glutamic Pyruvic Transaminase (GPT) and Glutamic Oxaloacetic Transaminase (GOT). While for renal toxicity, the following parameters were carried out: Alkaline Phosphatase (ALP), Acid Phosphatase (ACP), total protein content, total alpha amino acids, albumin and glucose. These parameters were assessed in urine samples, collected at monthly intervals. In liver, kidney and serum the four enzymes (GPT, GOT, ALP and ACP) and total protein content were estimated. Albumin estimation in serum and glucose in whole blood was done by using manufacturer's protocol. The parameters in tissue were done at the end of exposure period i.e. 120 days after sacrificing the animals by decapitation.

Statistical analysis

All the experimental results were expressed as the mean ± standard deviation. Unpaired T-test and one way analysis of variance (ANOVA) with subsequent Tukey's test were used to detect further difference between groups respectively, values of p< 0.05 were considered significant.

Results

Biochemical estimations

Urinary analysis: The activity and daily excretion of alkaline phosphatase (Table 1), Acid phosphatase (Table 2), Glutamic Pyruvic Transferase (GPT) (Table 3), Glutamic Oxaloacetic Transferase (GOT) (Table 4) in urine of Cd exposed rats of both dietary groups were significantly enhanced from day 30 of exposure onwards. These effects were generally more marked in the protein malnourished animals; here data of 120 days are shown. (p<0.005).

The urinary concentration of alpha-amino acids (Table 5), Albumin (Table 6), total protein (Table 7) and glucose (Table 8) was significantly enhanced in the Cd exposed animals of both the dietary groups, from day 30 of exposure onwards but the effect was more marked in the protein malnourished animals. The daily excretion was also significantly enhanced from day 30 to 120 of Cd exposure in the

Treatment	n mole p-nitro phenol formed/ml of urine				n mole p-nitro phenol formed/ml/day/rat			
	Days				Days			
	30	60	90	120	30	60	90	120
Normal diet	463.02 ± 42.32	551.82 ± 5.29	530.48 ± 6.77	520.35 ± 6.89	1393 ± 122	2436 ± 21	2466 ± 20	2420 ± 18
Normal diet + Cd	565.49 ± 16.51	760.82 ± 7.9	675.02 ±7.73	645.50 ± 7.90	1626 ± 57	3079± 76	3279± 87	3000 ± 25
	(↑ 22)ᵃ *	(↑ 38)ᵃ **	(↑ 27)ᵃ **	(↑ 24)ᵃ *	(↑ 17)ᵃ *	(↑ 26)ᵃ **	(↑ 33)ᵃ ***	(↑ 24)ᵃ **
Low protein diet	416.55 ± 50.78	601.1 ± 3.01	592.83 ± 9.76	535.75 ± 9.80	527 ± 46	820 ± 16	828 ± 23	810 ± 15
Low protein diet + Cd	579.0 ± 8.38	787.44 ± 2.0	847.75 ± 1.58	712.55 ± 1.60	643 ± 23	976 ± 5	1217 ± 26	1093 ± 8
	(↑ 39)ᵇ **	(↑ 31)ᵇ **	(↑ 43)ᵇ ***	(↑ 33)ᵇ **	(↑ 22)ᵇ **	(↑ 19)ᵇ *	(↑ 47)ᵇ ***	(↑ 35)ᵇ ***

Values represent mean ± SE of six rats; Statistical evaluation by one–way ANOVA followed by LSD comparison; a=Compared to normal diet control, b= Compared to low protein diet control; p * =<0.05; ** = 0.01; *** = <0.001; ↑=increase.

Table 1: Effect of Cd on urine levels of alkaline phosphatase in normal and protein malnourished rats (values in parentheses represent % change).

Treatment	n mole p-nitro phenol formed/ml of urine				n mole p-nitro phenol formed/ml/day/rat			
	Days				Days			
	30	60	90	120	30	60	90	120
Normal diet	172.55 ± 13.13	154.51 ± 5.12	156.81 ± 5.53	160.75 ± 5.60	961 ± 103	741 ± 35	773 ± 27	820 ± 29
Normal diet + Cd	151.29 ± 17.46	137.0 ± 3.2	191.31 ± 3.03	184.86 ± 3.42	788 ± 80	800 ± 52	1046 ± 43	955 ± 40
	(↓ 13)ᵃ *	(↓ 11)ᵃ **	(↑ 22)ᵃ **	(↑ 15)ᵃ *	(↓ 18)ᵃ *	(↑ 8)ᵃ **	(↑ 35)ᵃ ***	(↑ 16)ᵃ **
Low protein diet	160.09 ± 19.13	60.05 ± 2.5	63.45 ± 2.39	75.85 ± 2.54	325 ± 59	147 ± 3	230 ± 13	245 ± 15
Low protein diet + Cd	136.09 ± 6.61	71.26 ± 5.43	81.84 ± 2.97	105.44 ± 2.41	206 ± 7	158 ± 4	338 ± 15	314 ± 16
	(↓ 15)ᵇ NS	(↑ 19)ᵇ NS	(↑ 29)ᵇ **	(↑ 39)ᵇ ***	(↓ 37)ᵇ NS	(↑ 7)ᵇ NS	(↑ 47)ᵇ ***	(↑ 28)ᵇ **

Values represent mean ± SE of six rats; Statistical evaluation by one–way ANOVA followed by LSD comparison;
a = Compared to normal diet control, b= Compared to low protein diet control; p * =<0.05; ** = 0.01; *** = <0.001;
↑ =increase; ↓ = decrease, NS = Non-significant.

Table 2: Effect of Cd on urine levels of acid phosphatase in normal and protein malnourished rats (values in parentheses represent % change).

Treatment	n mole hydrazones formed/ml of urine				n mole hydrazones formed/ml/day/rat			
	Days				Days			
	30	60	90	120	30	60	90	120
Normal diet	31.472 ± 6.3	24.954 ± 6.35	20.67 ± 1.55	22.75 ± 1.60	127 ± 12	91 ± 5	76 ± 7	80 ± 6
Normal diet + Cd	38.759 ± 4.95	46.633 ± 2.11	28.31 ± 2.208	31.15 ± 2.21	173 ± 33	124 ± 5	84 ± 5	112 ± 7
	(↑ 23)[a] NS	(↑ 87)[a] **	(↑ 37)[a] *	(↑ 37)[a] *	(↑ 36)[a] NS	(↑ 36)[a] ***	(↑ 11)[a] NS	(↑ 40)[a] *
Low protein diet	25.89 ± 2.40	20.15 ± 3.53	20.01 ± 1.19	21.31 ± 1.20	63 ± 13	24 ± 2	25 ± 1	30 ± 3
Low protein diet + Cd	28.18 ± 43.078	73.078 ± 3.25	32.196 ± 3.02	36.456 ± 3.8	69 ± 22	56 ± 6	35 ± 6	45 ± 7
	(↑ 9)[b] NS	(↑ 114)[b] ***	(↑ 60)[b] ***	(↑ 71)[b] **	(↑ 10)[b] NS	(↑133)[b] ***	(↑ 40)[b] *	(↑ 50)[b] *

Values represent mean ± SE of six rats; Statistical evaluation by one–way ANOVA followed by LSD comparison;
a = Compared to normal diet control, b= Compared to low protein diet control; p * =<0.05; ** = 0.01; *** = <0.001;
↑ =increase; NS = Non-significant.

Table 3: Effect of Cd on urine levels of GPT in normal and protein malnourished rats (values in parentheses represent % change).

Treatment	n mole hydrazones formed/ml of urine				n mole hydrazones formed/ml/day/rat			
	Days				Days			
	30	60	90	120	30	60	90	120
Normal diet	31.472 ± 6.3	24.954 ± 6.35	20.67 ± 1.55	22.75 ± 1.60	127 ± 12	91 ± 5	76 ± 7	80 ± 6
Normal diet + Cd	38.759 ± 4.95	46.633 ± 2.11	28.31 ± 2.208	31.15 ± 2.21	173 ± 33	124 ± 5	84 ± 5	112 ± 7
	(↑ 23)[a] NS	(↑ 87)[a] **	(↑ 37)[a] *	(↑ 37)[a] *	(↑ 36)[a] NS	(↑ 36)[a] ***	(↑ 11)[a] NS	(↑ 40)[a] *
Low protein diet	25.89 ± 2.40	20.15 ± 3.53	20.01 ± 1.19	21.31 ± 1.20	63 ± 13	24 ± 2	25 ± 1	30 ± 3
Low protein diet + Cd	28.18 ± 43.078	73.078 ± 3.25	32.196 ± 3.02	36.456 ± 3.8	69 ± 22	56 ± 6	35 ± 6	45 ± 7
	(↑ 9)[b] NS	(↑ 114)[b] ***	(↑ 60)[b] ***	(↑ 71)[b] **	(↑ 10)[b] NS	(↑133)[b] ***	(↑ 40)[b] *	(↑ 50)[b] *

Values represent mean ± SE of six rats; Statistical evaluation by one–way ANOVA followed by LSD comparison;
a = Compared to normal diet control, b= Compared to low protein diet control; p * =<0.05; ** = 0.01; *** = <0.001;
↑ =increase; NS = Non-significant.

Table 4: Effect of Cd on urine levels of GOT in normal and protein malnourished rats (values in parentheses represent % change).

Treatment	g / 1				mg/day/rat			
	Days				Days			
	30	60	90	120	30	60	90	120
Normal diet	0.942 ± 0.053	0.962 ± 0.074	1.015 ± 0.094	1.002 ± 0.096	9.690 ± 0.16	10.013 ± 0.16	10.50 ± 0.40	10.60 ± 0.50
Normal diet + Cd	1.197 ± 0.084	1.331 ± 0.087	1.637 ± 0.094	1.488 ± 0.098	11.450 ± 0.29	11.653 ± 0.380	13.147 ± 0.563	13.988 ± 0.68
	(↑ 27)[a] **	(↑ 38)[a] ***	(↑ 61)[a] ***	(↑ 49)[a] **	(↑ 18)[a] ***	(↑ 16)[a] ***	(↑ 25)[a] ***	(↑ 32)[a] **
Low protein diet	0.532 ± 0.014	0.552 ± 0.013	0.57 ± 0.010	0.546 ± 0.011	1.053 ± 0.08	1.050 ± 0.100	1.106 ± 0.141	1.155 ± 0.251
Low protein diet + Cd	0.80 ± 0.033	0.832 ± 0.026	0.874 ± 0.032	0.935 ± 0.035	1.292 ± 0.13	1.440 ± 0.114	1.918 ± 0.322	1.930 ± 0.20
	(↑ 51)[b] **	(↑ 51)[b] **	(↑ 54)[b] **	(↑ 68)[b] ***	(↑ 23)[b] NS	(↑37)[b] NS	(↑ 73)[b] **	(↑ 69)[b] ***

Values represent mean ± SE of six rats; Statistical evaluation by one–way ANOVA followed by LSD comparison;
a = Compared to normal diet control, b= Compared to low protein diet control; p ** =<0.01; *** =< 0.001;
↑ =increase; NS = Non-significant.

Table 5: Effect of Cd on urine levels of total amino acids in normal and protein malnourished rats (values in parenthesis represent % change).

Treatment	g / 1				mg/day/rat			
	Days				Days			
	30	60	90	120	30	60	90	120
Normal diet	1.009 ± 0.08	2.013 ± 0.051	2.146 ± 0.05	2.146 ± 0.06	7.367 ± 1.39	7.493 ± 0.498	10.796 ± 0.25	10.695 ± 0.263
Normal diet + Cd	1.594 ± 0.173	3.133 ± 0.307	3.679 ± 0.244	3.671 ± 0.251	7.734 ± 1.013	8.372 ± 0.388	14.019 ± 0.07	14.00 ± 0.07
	(↑ 58)[a] **	(↑ 56)[a] ***	(↑ 72)[a] ***	(↑ 71)[a] ***	(↑ 5)[a] NS	(↑ 12)[a] NS	(↑ 30)[a] ***	(↑ 31)[a] ***
Low protein diet	0.983 ± 0.152	1.867 ± 0.049	1.910 ± 0.09	1.825 ± 0.09	3.455 ± 0.164	2.268 ± 0.110	4.264 ± 0.092	4.132 ± 0.099
Low protein diet + Cd	1.003 ± 0.060	2.109 ± 0.151	2.750 ± 0.060	3.157 ± 0.063	3.290 ± 0.269	4.142 ± 0.48	6.298 ± 0.12	6.399 ± 0.13
	(↑ 2)[b] NS	(↑ 13)[b] NS	(↑ 44)[b] ***	(↑ 73)[b] ***	(↑ 5)[b] NS	(↑83)[b] **	(↑ 48)[b] ***	(↑ 55)[b] ***

Values represent mean ± SE of six rats; Statistical evaluation by one–way ANOVA followed by LSD comparison;
a = Compared to normal diet control, b= Compared to low protein diet control; p ** =<0.01; *** =< 0.001;
↑ =increase; ↓ = decrease; NS = Non-significant.

Table 6: Effect of Cd on urine – albumin in normal and protein malnourished rats (values in parentheses represent % change).

normal diet fed rats. In the protein malnourished group, significant and more marked increase in the daily excretion of alpha-amino acids, albumin, total protein and glucose was observed on the day 90 and 120 of Cd exposure. A statistically non-significant increase was observed in these animals on days 30 and 60.

Tissue analysis

Effect on hepatic enzymes: A significant reduction in the activities of GPT, GOT and alkaline phosphatase was observed in the Cd exposed animals of both the dietary groups. The reduction in the GPT activity was more marked in the malnourished rats whereas the reductions

Treatment	g / 1				mg/day/rat			
	Days				Days			
	30	60	90	120	30	60	90	120
Normal diet	1-101 ± 0.098	2.390 ± 0.095	2.448 ± 0.095	2.475 ± 0.098	7.750 ± 1.418	7.769 ± 0.54	11.618 ± 4.269	11.712 ± 4.300
Normal diet + Cd	1.868 ± 0.216	3.537 ± 0.292	4.297 ± 0.271	4.583 ± 0.292	8.204 ± 1.129	9.322 ± 0.4	14.286 ± 0.125	14.685 ± 0.130
	(↑ 70)a **	(↑ 48)a ***	(↑ 76)a ***	(↑ 85)a ***	(↑ 6)a NS	(↑ 20)a *	(↑ 23)a ***	(↑ 25)a ***
Low protein diet	1.052 ± 0.152	2.109 ± 0.069	2.239 ± 0.107	2.252 ± 0.110	3.546 ± 0.132	2.348 ± 0.104	4.498 ± 0.038	4.499 ± 0.031
Low protein diet + Cd	0.975 ± 0.071	2.615 ± 0.159	3.201 ± 0.102	3.355 ± 0.104	3.272 ± 0.251	3.240 ± 0.211	6.479 ± 0.132	6.875 ± 0.130
	(↑ 7)b NS	(↑ 24)b **	(↑ 43)b ***	(↑ 49)b ***	(↑ 8)b NS	(↑ 38)b **	(↑ 44)b ***	(↑ 53)b ***

Values represent mean ± SE of six rats; Statistical evaluation by one–way ANOVA followed by LSD comparison;
a = Compared to normal diet control, b= Compared to low protein diet control; p ** =<0.01; *** =< 0.001;
↑ =increase; ↓ = decrease; NS = Non-significant.

Table 7: Effect of Cd on urine levels of protein in normal and protein malnourished rats (values in parentheses represent % change).

Treatment	g / 1				mg/day/rat			
	Days				Days			
	30	60	90	120	30	60	90	120
Normal diet	16.667 ± 0.715	14.075 ± 0.223	8.953 ± 0.371	9.530 ± 0.36	0.923 ± 0.166	0.574 ± 0.017	0.549 ± 0.056	0.563 ± 0.045
Normal diet + Cd	18.33 ± 1.668	26.467 ± 0.192	16.307 ± 0.696	17.15 ± 0.595	1.093 ± 0.172	0.898 ± 0.034	0.701 ± 0.046	0.810 ± 0.051
	(↑ 70)a **	(↑ 48)a ***	(↑ 76)a ***	(↑ 85)a ***	(↑ 6)a NS	(↑ 58)a *	(↑ 28)a ***	(↑ 44)a ***
Low protein diet	15.667 ± 0.494	12.033 ± 0.248	9.345 ± 0.346	11.535 ± 0.450	0.642 ± 0.06	0.262 ± 0.011	0.164 ± 0.008	0.120 ± 0.007
Low protein diet + Cd	17.233 ± 0.719	17.207 ± 0.184	15.146 ± 0.431	17.150 ± 0.500	0.683 ± 0.031	0.379 ± 0.005	0.272 ± 0.003	0.221 ± 0.004
	(↑ 10)b NS	(↑ 43)b ***	(↑ 62)b ***	(↑ 49)b ***	(↑ 6)b NS	(↑ 45)b **	(↑ 66)b **	(↑ 84)b ***

Values represent mean ± SE of six rats; Statistical evaluation by one–way ANOVA followed by LSD comparison;
a = Compared to normal diet control, b= Compared to low protein diet control; p ** =<0.01; *** =< 0.001;
↑ =increase; ↓ = decrease; NS = Non-significant.

Table 8: Effect of Cd on urine levels of glucose in normal and protein malnourished rats (values in parentheses represent % change).

observed in the alkaline phosphatase and GOT activities were more marked in the normal protein diet fed rats. The reduction in the acid phosphatase activity was not statistically significant in both the diet groups (Table 9).

Effect on renal enzymes: Cd exposure resulted in a significant reduction in the activities of GPT, GOT and alkaline phosphatase in both the dietary groups. The reduction in alkaline phosphatase activity was more marked in the malnourished rats and the magnitude of the effect GPT and GOT activities was more or less same in both the diet groups (Table 10).

Effect on serum enzymes: A significant increase in the serum GPT, GOT, alkaline phosphatase and acid phosphatase was observed in both the dietary groups. The effect on these enzymes was more marked in the protein malnourished animals (Table 11).

Effect on blood glucose: Cd exposure resulted in a significant increase in blood glucose levels, of more or less equal magnitude, in both the diet groups (Table 12).

Effect on serum albumin: A significant reduction of the same magnitude in serum albumin level was observed in the Cd- exposed animals of both the diet groups (Table 12).

Discussion

Hepatotoxicity and nephrotoxicity are among the most important manifestations of Cd exposure and related to the tissue concentration. The uptake, retention and toxicity of Cd greatly depend on the nutritional factors such as dietary proteins [16]. The present study shows that Cd exposure resulted in a significant retardation in body weight growth and induced enzymuria, proteinuria, aminoaciduria, glycosuria, hepato and renal damages and alteration in metabolism of essential trace elements.

Previous studies have indicated that the release of enzymes in urine after repeated parenteral administration of Cd to rat may reflect the development of renal damage caused by the accumulation of critical concentration of the metal [17]. Alkaline phosphatase may be particularly sensitive, since it is localized in the brush border of the proximal tubule of the rat, which is the site of Cd- induced injury [18]. In the present study, the activity and daily excretion of alkaline phosphatase in urine of Cd- exposed rats of both dietary groups were significantly enhanced from day 30 of exposure onwards. These effects were generally more marked in the protein malnourished animals. A significant increase in the urinary excretion of alkaline phosphatase, within 48hr of commencing Cd treatment has been reported [17]. Synthesis of renal Cd–thionein requires 48 hr. following exposure to Cd for maximum induction [19]. Thus in absence of adequate amounts of the binding protein, Cd may interact with some vital cellular components, though the exact mechanism is unknown. In the present finding, protein malnutrition might have contributed towards less synthesis of Cd- binding protein thionein, owing to the dearth of essential amino acids required for the synthesis. The Cd exposed animals of either dietary group exhibited an enhanced urinary acid phosphatase activity and excretion on days 90 and 120 of exposure and the effect was more marked in the protein malnourished rats. This increase is in conformity with the findings of Nomiyama [15].

Since the serum activities of enzymes were considerably increased, it manifests that this increase in enzyme activities in the urine resulted from enzymes released from injured organs other than the kidneys. Some researchers proved that the increase in enzyme activity in the urine results from the malfunctioning process of re-absorption at the tubules [20,21]. In our investigation, liver seemed to be protected from Cd- induced liver infarction till day 60, as the activity of GPT was found to be increasing in urine only after day 60 of Cd- exposure. Malnourished animals showed more marked effect with decreased

Treatment	n mole hydrazones formed /m/mg protein		n mole p–nitro phenol formed/m/mg protein	
	GPT	GOT	Alkaline phosphatase	Acid phosphatase
Normal diet	223.06 ± 12.25	217.28 ± 8.32	107.84 ± 6.02	531.49 ± 52.10
Normal diet + Cd	175.22 ± 9.70	190.23 ± 7.46	64.73 ± 4.12	452.98 ± 33.53
	(↓ 21)[a] ***	(↓ 12)[a] *	(↓ 40)[a] ***	(↓ 15)[a] NS
Low protein diet	74.76 ± 5.37	218.75 ± 8.76	108.56 ± 4.26	673.23 ± 59.49
Low protein diet + Cd	48.03 ± 2.55	207.54 ± 5.39	76.58 ± 0.41	599.11± 42.86
	(↓ 36)[b] *	(↓ 5)[b] NS	(↓ 29)[b] ***	(↓ 11)[b] NS

Values represent mean ± SE of six rats; Statistical evaluation by one–way ANOVA followed by LSD comparison;
a = Compared to normal diet control, b= Compared to low protein diet control; p ** =<0.05; *** =< 0.01;
↓ = decrease; NS = Non-significant.

Table 9: Effect of Cd exposure (120 days) on hepatic enzyme activity in normal and protein malnourished rats (values in parentheses represent % change).

Treatment	n mole hydrazones formed /m/mg protein		n mole p–nitro phenol formed/m/mg protein	
	GPT	GOT	Alkaline phosphatase	Acid phosphatase
Normal diet	33.80 ± 0.75	38.90 ± 0.64	392.17 ± 38.19	88.74 ± 6.72
Normal diet + Cd	30.60 ± 0.10	35.33 ± 0.59	454.91 ± 40.31	83.98 ± 10.61
	(↓ 8)[a] ***	(↓ 9)[a] **	(↓ 16)[a] *	(↓ 5)[a] NS
Low protein diet	36.92 ± 1.01	43.74 ± 0.58	520.31 ± 44.0	119.41 ± 9.83
Low protein diet + Cd	33.01 ± 0.65	40.98 ± 0.80	660.793 ± 43.65	116.05 ± 17.06
	(↓ 11)[b] **	(↓ 6)[b] **	(↓ 27)[b] **	(↓ 3)[b] NS

Values represent mean ± SE of six rats; Statistical evaluation by one–way ANOVA followed by LSD comparison;
a = Compared to normal diet control, b= Compared to low protein diet control; p ** =<0.05; *** =< 0.01;
↓ = decrease; NS = Non-significant.

Table 10: Effect of Cd exposure (120 days) on renal enzyme activity in normal and protein malnourished rats (values in parentheses represent % change).

Treatment	n mole hydrazones formed /m/mg protein		n mole p–nitro phenol formed/m/mg protein	
	GPT	GOT	Alkaline phosphatase	Acid phosphatase
Normal diet	32.44 ± 1.32	90.39 ± 1.08	43.07 ± 1.23	40.35 ± 0.49
Normal diet + Cd	50.79 ± 0.56	108.92 ± 0.86	55.75 ± 2.19	45-14 ± 1.45
	(↑ 57)[a] ***	(↑ 9)[a] **	(↑ 16)[a] *	(↑ 5)[a] NS
Low protein diet	30.56 ± 1.22	83.25 ± 2.23	40.86 ± 1.71	39.73 ± 0.52
Low protein diet + Cd	56.89 ± 1.67	123.59 ± 2.17	63.10 ± 1.02	58.45 ± 1.98
	(↑ 86)[b] ***	(↑ 48)[b] ***	(↑ 54)[b] ***	(↑ 47)[b] ***

Values represent mean ± SE of six rats; Statistical evaluation by one–way ANOVA followed by LSD comparison;
a = Compared to normal diet control, b= Compared to low protein diet control; p ** =<0.05; *** =< 0.01;
↓ = decrease; NS = Non-significant.

Table 11: Effect of Cd exposure (120 days) on Serum enzyme activity in normal and protein malnourished rats (values in parentheses represent % change).

Treatment	mg/dl	g/d1
	Blood glucose	Serum albumin
Normal diet	38.470 ± 0.59	3.776 ± 0.05
Normal diet + Cd	50.773 ± 1.25	3.383 ± 0.04
	(↑ 32)[a] ***	(↓10)[a] ***
Low protein diet	42.833 ± 2.15	3.303 ± 0.03
Low protein diet + Cd	54.609 ± 1.43	2.954 ± 0.09
	(↑ 27)[b] ***	(↓ 11)[b] ***

Values represent mean ± SE of six rats; Statistical evaluation by one–way ANOVA followed by LSD comparison;
a = Compared to normal diet control, b= Compared to low protein diet control; p ** =<0.05; *** =< 0.01;
↑ = increase; ↓ = decrease.

Table 12: Effect of Cd exposure (120 days) on Serum enzyme activity in normal and protein malnourished rats (values in parentheses represent % change).

rate of detoxification by Cd- thionein. Another most probable cause of enzymuria seems to be the release of enzymes from destroyed tubular cell which are rich in enzymes such as ALP, GOT and GPT. This observation is supported by the fact that renal tissue levels of ALP, GOT and GPT decreased significantly in Cd exposed rats of either dietary group. The significant inhibition in the activities of renal GOT and GPT upon Cd feeding is an indication of Cd nephrotoxicity [22]. High acid and alkaline phosphatase activities have been reported in rats fed on protein deficient diets [23]. It is highly probable that

increase in alkaline phosphatase contributes to retardation of growth in malnourished animals.

We found that the urinary concentration of amino acids was significantly enhanced in the Cd exposed animals of both the dietary groups, from day 30 of exposure onwards, but the effect was more marked in the protein malnourished animals. The daily excretion was also significantly enhanced from day 30 to 120 of Cd exposure in the normal diet fed rats. In the protein malnourished group, significant and more marked increase in the daily excretion of amino acid was

observed on the day 90 and 120 of Cd exposure. The present data may suggest that the critical concentration of cadmium in the renal cortex is lower for aminoaciduria than for proteinuria or glycosuria. Cd caused a dysfunction in amino acid nitrogen metabolism, such as increased glutamic acid metabolism, decreased urea synthesis and decreased ammonium formation have also been supported by previous finding [24]. On the basis of experiments on rabbits given subcutaneous injection of $CdCl_2$ at 1.5mg Cd/kg for 21 days, results showed aminoaciduria might be caused by disturbed tubular re-absorption of amino acids due to the increased clearance ratio of amino acids to creatinine [15]. The daily excretion of proteins in urine was also significantly enhanced from day 60 of Cd exposure in both the dietary groups, but the effect was more marked in protein malnourished animals.

In recent years, glomerular dysfunctions have been reported to be more prominent than tubular dysfunction in Cd intoxication [25,26]. The main protein components in urine of human beings and animals exposed to Cd were large molecular weight protein such as albumin; low molecular weight proteins were minor components, although they are specific for tubular disease. A decrease in protein intake leads to a rise in protein catabolism in liver [27] along with an increase in its hydrolytic activity. This supports the decreased levels of total protein and albumin in urine of protein malnourished rats.

Thus, the present study manifests that exposure to even low dose levels of Cd, coupled with protein under-nutrition increases the risk of environmental exposure of cadmium to man.

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