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Toxic Effects of Various Dietary Levels of Combined Cadmium Chloride and Zinc Chloride on Male Wistar Rats

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ABSTRACT

The present study was planned to elucidate the role of zinc in the modification of toxicity of cadmium with emphasis on vital organ dysfunction. Eighty, 3-months old male Wistar rats were fed cadmium chloride ($CdCl_2$) and Zinc chloride ($ZnCl_2$) mixture for 6 or 12 weeks. Group 2 (10 ppm $CdCl_2$ + 4 ppm $ZnCl_2$), group 3 (100 ppm $CdCl_2$ plus 100 ppm $ZnCl_2$) and group 1 was kept as control rats. The rats fed diet containing 10 ppm $CdCl_2$ plus 4 ppm $ZnCl_2$ of this mixture had the lowest growth rate after 12 weeks, but none of the rats died along the experimental period. Depression in growth, nephropathy, testicular injury and reduction in the size of the splenic white pulp were observed in group 2 and 3. Changes in serum alanine aminotransferase (ALT), aspartate aminotranferase (AST) and alkaline phosphatase (ALP) activities were significant increased in group 2 and 3. Alteration in concentration of total protein, albumin, cholesterol and urea were observed within these two treated groups. At 12 weeks, the values of erythrocytic series in rats of group 2 and 3 did not change. However, the values of platelets were higher in rats in group 2 and 3 then control (group 1). The values of WBC were higher in group 2 than control and group 3 and those of lymphocytes were lower in group 2 and 3 than control rats (group 1). The result indicated that Zn did not reverse the negative effects of Cd in growth and in organs damage particularly the kidneys, testes and spleen. This damage probably contributed to the increase in AST, ALT and ALP activities and cholesterol and urea concentrations with decrease in total protein and albumin concentrations.

Key words: Toxicity, nephropathy, testicular injury, Wistar rats

INTRODUCTION

Cadmium (Cd) is a heavy metal that is widely distributed in the environment and is present in trace levels in seawater and in a broad range of animal and plant species. Cd is a toxicologically important environmental contaminant, accumulates in the body and inhibits a number of enzymes containing sulfhydral groups. Relatively large quantities of Cd are found in commercial phosphate fertilizer, thus the increases in soil and plant Cd contents may lead to increases in dietary Cd. In recent years, Cd poses a potential environmental hazard due to increases in its industrial use (Valle and Ulmer, 1972; WHO, 1992). It was reported that the maximum tolerable dietary Cd level for domestic animals was 0.5 ppm. Dietary concentrations of 1 ppm results in undesirable effects while 5 ppm causes adverse health effects (McDowell, 1992).

Zinc is one of the nutrients that reduced the toxicity of orally consumed cadmium (Fox, 1987; McDowell, 1992). It competes cadmium for the same transport system as well for the binding site in metallothionein.

The high dietary levels of Cd results in suppressed feed intake and weight gain, reduction in bone mineralization and anaemia (Vallee and Ulmer, 1972; WHO, 1992). The biochemical alterations occur prior to morphological changes in the organs and the changes in certain enzyme levels in extracellular fluids may reflect the extent of Cd-induced damage in target organs (Khandelwal *et al*., 1991).

Zinc deficiency is markedly increases cadmium concentrations in various organs (Waalkes, 1986; Tanaka *et al*., 1995) and the accumulated cadmium disturbs zinc-dependent metabolic processes in the body. However, little is know about how zinc deficiency modifies cadmium toxicity (Sato and Nagai, 1989). It has been suggested that bone lesions are secondary changes due to renal failure and/ or nutritional imbalance. However, dietary cadmium did not cause osteomalacia even under seven zinc deficiency conditions (Tanaka *et al*., 1995). It was reported that Zn has a protective effect on hepatic cellular damage by maintaining membrane integrity due to its direct action on free radicals (Liu *et al*., 1992).

The present study was planned to elucidate the role of zinc in the modification of toxicity of cadmium with emphasis on vital organ dysfunction.

MATERIALS AND METHODS

Study design: Eighty, 3-months old male Wistar rats were housed within the premises of the Medicinal and Aromatic Plants Research Institute (MAPRI), National Center for Research, Khartoum, with feed and drinking water provided *ad libitum*. The rats were allotted at random to three groups, each of 6 rats. The study was conducted within the premises of the (MAPRI), National center for Research, Khartoum during 2008-2009.

Group 1 continued to be fed the normal diet and served as control. Cadmium Chloride and zinc chloride was thoroughly mixed with normal diet and fed to rats at 10 ppm $CdCl_2$ plus 4 ppm $ZnCl_2$ (group 2), 100 ppm $CdCl_2$ plus 100 ppm $ZnCl_2$ (group 3) for 6 or 12 weeks.

Average body weights and body weight gain were measured weekly for each group. After 6 or 12 weeks of treatment, rats from each group were killed under diethyl ether anesthesia to identify growth lesion and specimens of the liver, kidneys, spleen, heart and testes were immediately fixed in 10% neutral buffered formalin and processed for histopathology. Blood samples were collected from the blood vessels of each rat for serum analysis and hematology.

Blood analysis: Serum samples were analyzed for the activity of aspartate aminotransferase (AST), alanine aminotransferase (ALT) and alkaline phosphate (ALP) and for concentrations of total protein, albumin, globulin, bilirubin, cholesterol and urea.

Hemoglobin (Hb) concentration, Red Blood Cell (RBC) counts, Packed Cell Volume (PCV), Mean Corpuscular Volume (MCV), Mean Corpuscular Hemoglobin (MCH), Mean Corpuscular Hemoglobin Concentration (MCHC), White Blood Cell (WBC) counts and platelet counts were determined by Sysmex Kx-21 Analyzer (Japan, 1999) for haematological parameters.

Statistical analysis: The significant of differences between means was compared at each time point using Duncan's multiple range test after ANOVA for one-way classified data (Snedecor and Cochan, 1989).

RESULTS

Effect on growth: At 12 weeks, the rats in groups 2 and 3 that fed $CdCl_2$ + $ZnCl_2$ (10+4 ppm and 100+100 ppm) had significant low ($p<0.05$-0.001) growth rate than control (group 1) (Table 1). No change was observed in the control rats.

Pathological changes: At 6 weeks, the liver of rats fed 100 ppm $CdCl_2$+100 ppm $ZnCl_2$ (group 3) showed mild fatty cytoplasmic vaccuolation in the centrilobular zone of hepatocytes. The cells of the renal proximal convoluted tubules showed sever vacuolation or necrosis with glomerular alterations and acidophilic homogeneous material in the affected tubules. Reduction in the size of the splenic white pulp and testicular degeneration particularly of the epithelial cells of the seminiferous tubules were seen. In group 2 (4 ppm $CdCl_2$ + 10 ppm $ZnCl_2$), the liver lesions were also mild but those of the kidney were severe after 6 weeks. At 12 weeks, the liver had mild degenerative changes in the centrilobular cells but the kidney had severe vacuolation and necrosis of the proximal tubule. Marked reduction in the splenic white pulp was observed.

Hematological and serobiochemical changes: At 6 weeks there were no significant changes in erythrocytic series of rats in group 2 (4 ppm $CdCl_2$ + 10 ppm $ZnCl_2$) and 3 (100 ppm $CdCl_2$ + 100 ppm $ZnCl_2$) when compared with control (Group 1). Rats in group 2 had significant lower values ($p<0.05$) neutrophils and significant increase in lymphocytes ($p<0.05$) were recorded. The values of platelets were higher in group 2 ($p<0.05$) and lower ($p<0.05$) in group 3 than control. The activities of ALT were higher ($p<0.05$-0.001) in group 2 and 3 and those of ALP were higher ($p<0.05$) in group 3 than control and group 2. The concentrations of total protein and albumin were lower ($p<0.05$-0.001) in group 2 and 3, while, the concentration of bilirubin were higher ($p<0.01$-0.001) in the same groups than control (Group 1). The concentration of cholesterol and urea were also higher ($p<0.01$) in group 3 than control and group 2.

At 12 weeks, the values of erythrocytic series in rats of group 2 and 3 did not change. However, the values of platelets were higher ($p<0.05$-0.001) in rats in group 2 and 3 then control (Group1). The value of WBC were higher ($p<0.01$) in group 2 than control and group 3 and those of lymphocytes were lower ($p< 0.05$) in group 2 and 3 than control (Group 1).

The activities of ALT were still higher ($p<0.01$-0.001) in group 2 and 3 and those of ALP were higher ($p<0.05$) in groups 2 and 3 than control. The activities of AST was only higher in group 3 ($p<0.05$) than control and group 2. The concentrations of total protein and albumin were lower ($p<0.05$-0.01) in group 3 and those of bilirubin were higher ($p<0.05$) in the same group than control and group 2 (Table 2, 3).

Table 1: Changes in body weights and body weight gains in rats fed diets containing various levels of cadmium chloride and zinc chloride mixtures for 12 weeks

Treatment group	Body weight (g)	Body weight gain (g)
6 weeks		
Control (normal diet)	139±3.7	61±2.0
$CdCl_2$ + $ZnCl_2$ (10+4 ppm)	139±3.4^{NS}	63±2.2^{NS}
$CdCl_2$ + $ZnCl_2$ (100+100 ppm)	134±3.2^{NS}	61±2.3^{NS}
12 weeks		
Control (normal diet)	189±4.6	50±2.2
$CdCl_2$ + $ZnCl_2$ (10+4 ppm)	143±2.2*	4±0.5***
$CdCl_2$ + $ZnCl_2$ (100+100 ppm)	162±1.8*	28±1.8**

Values are Mean±SE. NS: Not significant; *$p<0.05$; **$p<0.01$; ***$p<0.001$

Table 2: Haematological changes in rats fed diets containing various levels of cadmium chloride and zinc chloride mixtures for 12 weeks

Parameters	Control (normal diets)	$CdCl_2$+$ZnCl_2$ (10+4 ppm)	$CdCl_2$+$ZnCl_2$ (100+100 ppm)
6 weeks			
Hb (g dL^{-1})	13.4±1.9	14.3±2.1^{NS}	14.4±1.8^{NS}
RBC (106 mm^3)	7.0±1.1	7.1±1.8^{NS}	6.8±1.6^{NS}
PCV (%)	41.3±3.3	42.0±3.2^{NS}	44.0±3.1^{NS}
MCV (m^3)	58.7±2.2	59.7±2.6^{NS}	64.3±2.4^{NS}
MCHC (%)	32.5±1.8	33.9±1.6^{NS}	32.8±1.6^{NS}
MCH (pg)	19.1±1.4	20.2±1.2^{NS}	21.1±1.1^{NS}
PLT (10^3 mm^3)	672.7±7.7	754.3±3.8*	565.0±3.9*
WBC (10^3 mm^3)	4.5±1.3	4.9±1.1^{NS}	4.34±1.5^{NS}
Neuts (%)	37.3±1.6	25.3±1.2*	30.0±1.5^{NS}
Lymphs (%)	62.7±2.1	74.7±0.5*	70.0±1.2^{NS}
12 weeks			
Hb (g dL^{-1})	13.4±1.9	14.2±2.2^{NS}	15.0±2.3^{NS}
RBC (10^6 mm^3)	7.0±1.1	8.9±1.3^{NS}	9.6±1.1^{NS}
PCV (%)	41.3±3.3	40.7±2.6^{NS}	33.7±2.1^{NS}
MCV (m^3)	59.0±2.4	49.7±3.1^{NS}	35.3±4.2^{NS}
MCHC (%)	32.6±1.5	35.6±1.4^{NS}	44.6±3.6^{NS}
MCH (pg)	19.2±1.5	16.0±1.3^{NS}	15.6±1.5^{NS}
PLT (10^3 mm^3)	672.7±7.7	700.0±3.8*	736.0±3.5**
WBC (10^3 mm^3)	4.5±1.3	8.0±1.3**	5.6±1.1^{NS}
Neuts (%)	37.3±1.6	52.0±0.9*	48.0±0.8*
Lymphs (%)	62.7±2.1	48.0±0.5*	52.0±0.6*

Values are Mean±SE. NS: Not significant; *p<0.05, **p<0.01, ***p<0.001

Table 3: Serobiochemical changes in rats fed diets containing various levels of cadmium chloride and zinc chloride mixtures for 12 weeks

Parameters	Control (normal diet)	$CdCl_2$+$ZnCl_2$ (10+4 ppm)	$CdCl_2$+$ZnCl_2$ (100+100 ppm)
6 weeks			
AST (iu)	39.6±1.90	40.7±1.80^{NS}	55.0±0.40*
ALT (iu)	16.3±1.10	48.6±0.70**	70.3±1.20***
ALP (iu)	139.0±4.00	111.0±1.10*	152.7±2.10*
T. protein (g dL^{-1})	7.2±0.70	5.7±0.40*	5.1±0.90*
Albumin (g dL^{-1})	4.6±0.50	3.2±0.09**	2.7±0.07***
Globulin (g dL^{-1})	2.6±0.40	2.5±0.40^{NS}	2.4±0.50^{NS}
Bilirubin (mg dL^{-1})	0.9±0.09	1.8±0.10**	2.3±0.10***
Cholesterol (mg dL^{-1})	196.7±6.70	191.0±6.20^{NS}	287.0±4.60**
Urea (mg dL^{-1})	46.3±3.90	53.0±2.10^{NS}	62.0±1.00**
12 weeks			
AST (iu)	39.6±1.90	42.3±1.80^{NS}	51.0±0.60
ALT (iu)	18.3±1.20	45.0±1.70**	95.0±1.60***
ALP (iu)	136.0±1.30	163.3±1.40**	220.7±2.30***
T. protein (g dL^{-1})	7.3±0.70	7.0±0.60^{0NS}	5.3±0.50*
Albumin (g dL^{-1})	4.3±0.60	4.5±0.50^{NS}	2.8±0.07**
Globulin (g dL^{-1})	3.0±0.40	2.5±.060^{NS}	2.5±0.50^{NS}
Bilirubin (mg dL^{-1})	0.9±0.09	0.6±0.08^{NS}	1.4±0.07*
Cholesterol (mg dL^{-1})	196.7±6.70	17.0±2.40*	178.0±4.90^{NS}
Urea (mg dL^{-1})	46.3±3.90	53.3±2.10^{NS}	54.3±2.20^{NS}

Values are Mean±SE. NS: Not significant; *p<0.05; **p<0.01; ***p<0.001

DISCUSSION

Cadmium toxicity is directly related to Cadmium absorption and retention in some tissues such as liver, kidney and gastrointestinal tract.

Trace mineral nutrition is the most important factor affecting Cd absorption (Van Bruwaen *et al*., 1984). Increasing Zn consumption will lower Cd uptake and retention (Bremner, 1978). The reduction of Cd toxicity by Zn administration is thought related to metallothionein synthesis (Webb, 1972; Leber and Miya, 1976). In the presented study, live weight of the birds was significantly lower than the controls; this is consistent with the findings as in mice (Malave and De Ruffino, 1984), rats (Rajanna *et al*., 1984), leghorn chicks (WHO, 1992) and in broilers (Uyanik *et al*., 2001). Although, feed consumption was not recorded, a reduction was observed in treatment groups as indicated by others (WHO, 1992), the lowered live weight may result from the reduced feed intake.

In the present investigation, rats fed combined levels of Cadmium chloride and zinc chloride had a little or diminished pathological effect on liver but not on kidney, testis or spleen. It has been previously suggested that zinc has a protective effects on hepatic cellular damage by maintaining membrane integrity due to indirect action on free radical (Liu *et al*., 1992).

Zinc given prior Cadmium or Cadmium-metallothionein administration may prevent nephrotoxic impact of Cadmium with out decrease kidney-Cadmium level (Dorian and Klaassen, 1995; Tang *et al*., 1998) and the protective effect of zinc was a result of increased synthesis of metallothionein with the pre-treatment.

In the present study, increases in AST, ALT and ALP levels (Uyanik *et al*., 2001) may result from the liver damage as supported by the pathological findings, consistent with the findings of Saygy *et al*. (1991) and Uyanik *et al*. (2001). A slight but not significant increase in ALP activity in agreement with the finding of Khandelwal *et al*. (1991) and Uyanik *et al*. (2001) may result from poor growth.

In conclusion of the present study, the result indicated that Zn did not reverse the negative effects of Cd in growth and in organs damage particularly kidney, testes and spleen. This damage probably contributed to increase AST, ALT and ALP activities and cholesterol and urea concentrations with decrease in total protein and albumin concentrations consistent with the findings of Uyanik *et al*. (2001).

REFERENCES

Bremner, I., 1978. Cadmium toxicity. World Rev. Nutr. Dietetic, 32: 156-197.

Dorian, C. and C.D. Klaassen, 1995. Protection by zinc-metallothionein against cadmium-metallothionein in nephrotoxicity. Toxicol. Sci., 26: 99-106.

Fox, M.R.S., 1987. Assessment of cadmium, lead and vanadium status of large animals as related to human food chain. J. Anim. Sci., 65: 1744-1752.

Khandelwal, S., N. Agnihotri and S.K. Tandon, 1991. Biochemical response to cadmium: Dose-time effect. Biol. Trace Elem. Res., 29: 157-164.

Leber, A.P. and T.S. Miya, 1976. A mechanism of cadmium and zinc induced tolerance to cadmium toxicity involvement of metallothiorein. Toxicol. Applied Pharmacol., 37: 403-414.

Liu, X.Y., T.Y. Jin, G.F. Nordberg, S. Ranner, M. Sjostrom and Y.A. Zhou, 1992. Multivariate study of protective effect of zinc and copper against nephrotoxcity induced by Cadmium matallothionein in rats. Toxicol. Applied Pharmacol., 114: 239-245.

Malave, I. and D.T. De Ruffino, 1984. Altered immune response during cadmium administration in mice. Toxicol. Applied Pharmacol., 74: 46-56.

McDowell, L.R., 1992. Minerals in Animal and Human Nutrition. Academic Press, San Diego, California.

Rajanna, B., M. Hobson, J. Reese, E. Sample and K.D. Chapatwala, 1984. Chronic hepatic and renal toxicity by cadmium in rats. Drug. Chem. Toxicol., 7: 229-241.

Sato, M. and Y. Nagai, 1989. Effect of zinc deficiency on the accumulation of metallothionein in the rate liver and kidney. Arch. Environ. Contamination Toxicol., 18: 587-593.

Saygy, S., G. Deniz, O. Kutsal and N. Vural, 1991. Chronic effects of cadmium on kidney, liver, testis and fertility of male rats. Biol. Trace Elem. Res., 31: 209-214.

Snedecor, G.W. and W.G. Cochan, 1989. Statistical Methods. 8th Edn., Iowa State University Press, Ames, IA., pp: 503.

Tanaka, M., M. Yanagi, K. Schirota, Y. Une, Y. Nomura, T. Masaoka and F. Akahorim, 1995. Effect of cadmium in zinc deficient rats. Vet. Hum. Toxicol., 37: 203-208.

Tang, W., S. Sadovic and Z.A. Shaikh, 1998. Nephrotoxicity of cadmium–metallothionein protection by zinc and glutathione. Toxicol. Applied Pharmacol., 15: 276-282.

Uyanik, F., M. Eren, A. Atasever, G. Tuncoku and A.H. Kolsuz, 2001. Changes in some biochemical parameters and organs of broilers exposed to cadmium and effect of Zinc on cadmium induced alteration. Israel J. Vet. Med., 56: 128-134.

Valle, B.L. and D.D. Ulmer, 1972. Biochemical effects of mercury, cadmium and lead. Annu. Rev. Biochem., 41: 91-128.

Van Bruwaen, R., R. Kirchmann and R. Impens, 1984. Cadmium contamination in agriculture and zoo technology. Experientia, 40: 43-50.

WHO, 1992. Cadmium. International programme on chemical safety. Environmental Health Criteria, pp: 134.

Waalkes, M.P., 1986. Effect of dietary zinc deficiency on the accumulation of Cadmium and metallothionein in selected tissues of rats. J. Toxicol. Environ. Health, 18: 301-313.

Webb, M., 1972. Protection by zinc against cadmium toxicity. Biochem. Pharmacol., 21: 2767-2771.