Journal of Nutritional & Environmental Medicine
September–December 2007; 16(3–4): 267–274

ORIGINAL RESEARCH

Oral cadmium exposure alters haematological and liver function parameters of rats fed a Nigerian-like diet

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Abstract
Purpose. The effect of oral cadmium toxicity on aspartate aminotransferase (AST) and alanine aminotransferase (ALT) activities and some haematological parameters was studied in Wistar albino rats fed a Nigerian-like diet (NLD) for 16 weeks.
Design. Two groups of rats were fed with either a normal diet or a NLD. Half of the animals in each group received deionized water and the other half received 100 mg cadmium kg^{-1} in drinking water. The plasma and liver activities of AST and ALT were assayed.
Methods. ALT, AST and haematological parameters were determined by standard procedures.
Results. There was a significant ($p<0.05$) increase in plasma AST and ALT and a corresponding decrease in the same enzymes in the liver of rats fed with a NLD and those exposed to cadmium. The NLD significantly reduced haemoglobin, haematocrit and red blood cell counts compared with the control diet and cadmium elaborated these effects.
Conclusion. This study shows that the NLD may predispose rats to liver and haematological dysfunction in cadmium toxicity.

Key words: *Cadmium, Nigerian-like diet, liver function, haematological parameters*

Introduction

Cadmium is a ubiquitous non-essential metal that has a long biological half-life. It contaminates water and food and with age it accumulates in human tissues, especially the liver and kidney, causing their damage [1]. The release of cadmium into the environment increased considerably in most industrialized countries during the second half of the last century. This has led to increased danger to health, not only in exposed workers, but also in the general population of these countries [1]. The uptake, distribution and toxicity of cadmium are influenced by the type or composition of the diet of a population. The studies of Anderson et al. [2] also indicate that semi-purified diets (low in fibre content and very much closer to a 'western human diet') result in increased cadmium uptake in mice

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ISSN 1359-0847 print/ISSN 1364-6907 online
DOI: 10.1080/13590840701775403

compared with unrefined whole diets rich in natural fibres, chelating agents and trace elements. Also, diets low in protein have been reported to increase the absorption of cadmium [3].

Diets low in proteins and high in carbohydrates have previously been shown to induce anaemia [4] and anaemia is a very common manifestation in chronic cadmium intoxication. The mechanism by which cadmium results in anaemia is still under investigation. However, some of the proposed mechanisms include decreased iron absorption, distortion of erythropoiesis and haemolysis of red blood cells [5,6].

Also, available reports indicate that cadmium-induced toxicity results from the generation of free radicals [7,8], which leads to lipid peroxidation, causing damage to many systems and organs and a change in their functions and structure [9]. The liver is one of the most susceptible organs after acute or chronic exposure to cadmium. Cadmium-induced damage of the liver is manifested by an increase in aspartate aminotransferase (AST) and alanine aminotransferase (ALT) in the blood [9,10].

The upsurge in the rate of industrial development in Nigeria is thought to be linked with the increasing levels of cadmium and other heavy metals in the environment [11]. The typical Nigerian diet is low in protein, but high in carbohydrate and fibre. Reports on the role of diet or its components on cadmium-induced anaemia and liver damage are lacking. There are even fewer still on the effect of a Nigerian diet. The present study examined the effect of a wholly compounded Nigerian-like diet (NLD) on liver function and haematological indices of rat after oral cadmium exposure.

Materials and methods

Materials

The reagents used in this study were of analytical grade from the British Drug House.

Experimental design

Forty male albino rats (Wistar strain) with an average weight of 100 ± 2 g were housed individually in stainless steel cages with wire mesh floors to prevent coprophagy. The rats were assigned to two diet groups with 20 animals each such that the weight difference between the groups was less than 0.2 g. One group was maintained on a NLD, which was low in protein and high in carbohydrates and fibre [12]. Fish, being one of the most important sources of protein in southern Nigeria, was used in compounding the NLD. The other group was maintained on a control diet. The composition of both diets is shown in Table I. The rats of each diet group were further assigned into two subgroups of 10 rats each, with one of these subgroups receiving 100 ppm cadmium ion (as $3CdSO_4.8H_2O$) in their drinking water. A preliminary investigation in our laboratory had established that this dose of cadmium ion was tolerated by the rats with quantifiable tissue biochemical changes without fatality.

The animals were acclimatized with these respective diets and water for 1 week before the commencement of the study, which was for 16 weeks. During the study period, water consumption, food intake and dry faecal output were recorded weekly. At the end of the study period, the animals were fasted for 18 hours and sacrificed after chloroform anaesthesia. All of the animal treatments were carried out in accordance with the principles of laboratory animal care of the NIN guide for laboratory animal welfare.

Table I. Percentage composition of the diets.

Dietary component	Control diet	Nigerian-like diet
Protein (defatted fish)	–	14.00
Protein (casein)	19.00	–
Sucrose	15.00	–
Garri	–	65.00
Corn starch	50.65	–
Fibre	–	10.65
Cellulose	5.00	–
Salt mix	4.00	4.00
Vitamin mix	1.00	1.00
Palm oil	5.00	5.00
Methionine	0.35	0.35
Sulphur content	0.25	0.16

Garri is a cassava-based meal commonly consumed in Nigeria. Garri contributed to the fibre in the Nigerian-like diet.

Collection and treatment of samples

While under chloroform anaesthesia, the left common carotid artery was exposed and cannulated. A blood sample was obtained from each rat via the cannula and a portion was transferred to a heparinized tube. Plasma was obtained by centrifugation of the blood at 3000*g* for 10 min and subsequently used for the analysis of the activities of ALT and AST. The remaining portion of blood was transferred to ethylenediaminetetraacetic acid (EDTA) containers and was later used for the analysis of haematological values. The abdominal cavity of each rat was also opened with a pair of scissors and the liver was excised and analysed for the above enzymes as well as the cadmium content. Analysis of the enzymes was carried out in 10% v/w liver homogenates. All of these analyses were carried out within 24 hours after sacrifice of the rats.

Biochemical analysis

The activities of ALT and AST were determined in the plasma and liver using the methods of Annino & Giese [13].

Haematological analysis

Red blood cell counts and white blood cell counts were determined in blood using a cell coulter T_{540}. The packed cell volume and haemoglobin concentration of each blood sample were determined by standard procedures.

Cadmium analysis

For the cadmium analysis, 1 g of liver was put into a beaker containing 20 ml acid mixture (HNO_3/$HClO_4$; 4:1; v/v) followed by heating at 100°C to facilitate digestion. The digests were allowed to cool and thereafter diluted with deionized water to give a final volume of 100 ml. The cadmium concentrations in the digests were measured by atomic absorption spectrophotometry using a Varian AA 1475 spectrophotometer.

Statistical analysis

The values are reported as mean ± standard error of the mean. Statistical difference was determined using analysis of variance and differences in the means were tested by Duncan's multiple range test [14].

Results

The body weight gain, feed and water intake and dry faecal output data of the rats in the experimental groups are presented in Table II. The results obtained indicate a significantly ($p<0.05$) decreased body weight gain and water intake of rats exposed to 100 ppm cadmium in drinking water relative to those given cadmium-free water. A significant ($p<0.05$) decrease in body weight gain and feed efficiency was found in rats fed the NLD, whereas the faecal output was significantly increased. The study shows that cadmium and the NLD decreased body weight gain and feed efficiency, but increased the faecal output of rats.

The data in Table III give the results of selected biochemical parameters and the liver cadmium content of rats in the experimental groups. The concentration of cadmium in the liver of cadmium-treated rats was significantly ($p<0.05$) increased relative to their controls. However, a point worth noting is the decreased accumulation of cadmium in the liver of rats fed the NLD and oral cadmium. Thus, the study indicates that the NLD affects the cadmium concentration in the liver in a manner that might suggest that it can protect rats in oral cadmium intoxication. The results obtained for plasma ALT and AST indicate that rats exposed to oral cadmium had significantly increased activity of those enzymes relative to rats given deionized water. The NLD also significantly increased plasma ALT and AST as compared with the control diet and the levels of the enzymes were even higher than those of rats fed the control diet and oral cadmium. The effect of the NLD on plasma ALT and AST was further enhanced in the presence of cadmium. Conversely, statistical evaluation of the data of liver ALT and AST of rats indicates a significant decrease in rats fed NLD compared with the control, a pattern that was observed in cadmium-exposed rats. However, the least activity of ALT and AST was observed in rats fed NLD plus oral cadmium. The study shows that plasma and liver aminotransferases were responsive to diet type and cadmium administration.

Table II. Weight gain, feed consumption, water intake and dry faecal output of rats in the experimental groups.

Parameter	Control diet	Control diet and cadmium	Nigerian-like diet	Nigerian-like diet and cadmium
Weight gain ($g\,day^{-1}\,rat^{-1}$)	1.69 ± 0.21^{a}	0.96 ± 0.05^{b}	1.01 ± 0.14^{b}	0.62 ± 0.06^{c}
Feed consumption ($g\,day^{-1}\,rat^{-1}$)	$13.08 \pm 1.14^{a,b}$	$11.04 \pm 1.23^{a,b}$	15.42 ± 1.02^{a}	$13.81 \pm 1.81^{a,b}$
Water intake ($ml\,day^{-1}\,rat^{-1}$)	43.50 ± 3.50^{a}	34.61 ± 2.00^{b}	49.06 ± 3.41^{a}	36.02 ± 2.10^{b}
Feed efficiency ($g\,body\text{-}weight^{-1}\,g\,feed^{-1}$) × 10	1.29 ± 0.02^{a}	0.87 ± 0.02^{b}	0.65 ± 0.02^{c}	0.47 ± 0.02^{d}
Dry faecal output ($g\,day^{-1}\,rat^{-1}$)	1.24 ± 0.03^{a}	1.70 ± 0.03^{b}	2.06 ± 0.02^{c}	2.62 ± 0.02^{d}

Values are means ± standard error of the mean (n = 10). Means of the same row followed by different letters differ significantly ($p<0.05$).

Table III. Effect of oral cadmium exposure on plasma, liver alanine aminotransferase (ALT) and aspartate aminotransferase (AST) activities and liver cadmium concentration of rats fed the Nigerian-like diet.

Parameter	Control diet	Control diet and cadmium	Nigerian-like diet	Nigerian-like diet and cadmium
Plasma aminotransferase				
ALT (units ml^{-1})	46.7 ± 4.1^{a}	51.3 ± 8.2^{b}	87.3 ± 5.0^{c}	149.3 ± 10.3^{d}
AST (units ml^{-1})	256.0 ± 17.5^{a}	325.3 ± 21.5^{b}	384.0 ± 12.3^{c}	609.5 ± 50.6^{d}
Liver aminotransferase				
ALT	5567.4 ± 113^{a}	4914.1 ± 210^{b}	4904.6 ± 106^{c}	4797.8 ± 150^{d}
AST	6564.8 ± 130^{a}	5307.0 ± 170^{b}	5087.8 ± 160^{c}	4010.0 ± 245^{d}
Cadmium concentration				
Liver ($\mu g\, g^{-1}$)	3.1 ± 0.09^{a}	50.5 ± 1.43^{b}	2.9 ± 0.03^{a}	37.2 ± 0.91^{c}

Values are means $\pm$ standard error of the mean (n=10). Means of the same row followed by different letters differ significantly ($p<0.05$).

The haemoglobin, haematocrit and red blood cell counts of rats maintained on the NLD were significantly decreased as compared with the controls. This effect was potentiated when the NLD-fed rats were given cadmium. No significant changes ($p>0.05$) in these parameters were observed in rats fed the control diet in addition to oral cadmium relative to those fed the control diet alone. The white blood cell count was not significantly altered in all of the experimental groups. Thus, the study shows that the NLD influenced the effect of cadmium on some haematological parameters.

Discussion

Exposure to cadmium is well known to induce a variety of toxicity symptoms in both experimental animals and exposed populations. The present study was prompted by the recent attention to the role of diet in cadmium toxicity. Our findings show that the diet reduced the weight gain of rats (Table II). Available reports indicate that a low protein, high carbohydrate, high fibre diet decreases weight gain [15]. Thus, the high fibre content of the NLD might have contributed to the observed decrease in body weight gain of rats fed this diet. The effect of cadmium on body weight gain observed in this study is consistent with previous reports [6,16]. Weight gain is influenced by the availability and absorption of nutrients. Recent studies show that cadmium decreases nutrient digestion and absorption [17,18]. Thus, the further reduction in body weight gain observed in rats fed the NLD in addition to oral cadmium (Table II) may be an indication of an additive effect.

Previous reports show that a high carbohydrate, low protein diet can lead to an increase in faecal bulk [19]. The low protein and high carbohydrate and fibre of the NLD may account for the observed increase in faecal output (Table II). The increased faecal bulk observed with cadmium administration is not unconnected to its effect on nutrient digestion and absorption.

Despite the duration of our study (16 weeks), the level of cadmium in the liver of rats exposed to NLD and oral cadmium was similar to that observed by Tewari et al. [3], who also exposed rats to 100 ppm cadmium in the diet for a shorter duration (8 weeks). It follows therefore that the NLD decreased cadmium bioaccumulation in the liver. This finding might be related to the high fibre content of the NLD. Dietary fibre has been reported to have an inhibitory effect on the gastrointestinal absorption of cadmium [20]. Moreover, it has been shown that after absorption of cadmium, it is delivered to the liver by endogenous intestinal metallothionein (MT). Therefore, cadmium absorption and transport is dependent on the protein MT because it aids these processes. Thus, the less marked accumulation of cadmium in the liver of rats fed the NLD and oral cadmium might also relate to decreased MT synthesis occasioned by low availability of dietary proteins and sulphur. MT has been shown to contain about 30% cysteine, a sulphur-containing amino acid [21].

Elevations in plasma aminotransferase activity have been reported to accompany damage to the liver and other tissues [13]. Also, an increase in free radical generation may occur under conditions of stress, such as malnutrition [22]. Free radical-induced oxidative stress causes membrane lipid peroxidation [23], which may result in tissue damage and leakage of enzymes. Consequently, the elevated plasma ALT and AST and the corresponding reduction in enzymes in the liver of rats fed NLD (Table III) is probably an indication of liver damage occasioned by lipid peroxidation. Haemoglobin synthesis would probably be depressed in animals fed a low protein diet, thus the low haemoglobin level observed in the rats fed with the NLD may in part be due to the low protein content of the diet. Likewise, the absorption, storage and transport of iron (needed for haemoglobin synthesis) from the gastrointestinal tract also require the proteins transferin and ferritin, so the synthesis of these proteins will be reduced in the NLD-fed rats, which would also contribute to the observed decrease in the haematological parameters relative to the rats fed the control diet (Table IV).

Generally the liver is one of the critical target organs after acute and chronic exposures to cadmium [10,24]. Also, cadmium can induce lipid peroxidation in tissues [8], which might lead to necrosis. Cadmium-induced necrosis in the liver can cause the release of abnormal quantities of L-ALT and L-AST into the blood. As in rats fed the NLD, the significantly increased plasma L-ALT and L-AST activities and the corresponding decrease in these enzymes in the liver (Table III) are probably an indication of liver damage induced by cadmium.

Of considerable interest in our investigation is the observed decrease in cadmium in the liver of rats fed the NLD in addition to cadmium. This finding might suggest that the NLD can protect rats in oral cadmium toxicity. However, examination of the data obtained for haematological and liver function parameters indicated that the NLD aggravated the effect of cadmium. This is evident when the results obtained in rats maintained on the NLD plus oral cadmium are compared with those of rats on the control diet plus oral cadmium. The aggravation of cadmium toxicity by the NLD may be due to a decrease in the synthesis of glutathione [25] and MT, as postulated by Tewari et al. [3]. MT sequesters cadmium and thus reduces its toxicity [26,27]. Thus, we believe that the effect of glutathione and MT

Table IV. Effect of oral cadmium exposure on haematological parameters of rats fed the Nigerian-like diet.

Parameter	Control diet	Control diet and cadmium	Nigerian-like diet	Nigerian-like diet and cadmium
Haemoglobin (g 100 ml^{-1})	15.3 ± 0.4^a	14.9 ± 1.2^a	10.2 ± 0.8^b	6.4 ± 0.7^c
Haematocrit (vol %)	44.9 ± 1.7^a	43.8 ± 2.7^a	29.7 ± 2.1^b	19.1 ± 1.2^c
Red blood cell count ($\times 10^6$ mm^{-3})	7.5 ± 1.4^a	7.4 ± 1.3^a	4.9 ± 0.5^b	2.9 ± 0.3^c
White blood cell count ($\times 10^2$ mm^{-3})	51.0 ± 9.0^a	42.5 ± 10.3^a	38.0 ± 7.0^a	43.2 ± 9.1^a

Values are means ± standard error of the mean (n = 10). Means of the same row followed by different letters differ significantly ($p < 0.05$).

would be higher in rats maintained on the control diet compared with rats on the NLD, which is low in protein and sulphur-containing amino acids. This hypothesis is particularly strengthened by the lack of a significant decrease in haematological values of rats fed the control diet in addition to cadmium exposure as compared with rats fed the control diet alone, whereas these values were significantly reduced in NLD plus oral cadmium-exposed rats (Table III).

In conclusion, our results indicate that the NLD rendered rats more susceptible to the effects of cadmium on the liver and the haematopoietic system. However, the precise mechanism of action needs to be further investigated.

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